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Genetic variation in physiology and growth under ambient and elevated CO2 concentrations in four provenances of Populus tremuloides from Northwestern Ontario

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Genetic variation in physiology and growth under ambient and elevated CO_2 concentrations in four provenances of *Populus tremuloides* from northwestern Ontario

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ABSTRACT

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Keywords: Provenance, family, leaf gas exchange, biomass allocation, trembling aspen, elevated CO_2.

Trembling aspen (*Populus tremuloides* Michx.) is the most widely distributed and probably most genetically varied woody species in North America. Four provenances of trembling aspen seedlings from northwestern Ontario were grown in the greenhouse to investigate genetic variation of growth and ecophysiological responses to ambient and elevated CO_2 concentrations. Two provenances were from southwest of Thunder Bay, and the other two were from north shore of Lake Superior. Three families per provenance were grown. Leaf gas exchange variables, growth and biomass were measured at 3 and 5 months old (August and October 2001) in the first year and 60 days after bud flush in the second year (April 2002). Significant differences between provenances were found in root collar diameter and height in the August 2001 measurement, and in total and stem biomass harvested in April 2002. There were no significant differences in leaf gas exchange or other biomass components expressed between provenances in April 2002. However, the seedlings expressed substantial family differences in leaf gas exchange, growth, biomass and biomass allocation variables. There were positive but low correlations between photosynthetic water use efficiency (WUE) and height and total biomass. Furthermore, provenance performance was predicted by most monthly climate variables suggesting adaptation of provenances to local climate. High family and single tree heritability estimates of biomass variables were present in the August 2001 measurement.

The seedlings were also exposed to three CO_2 concentrations (ambient, 540 PPM and 720 PPM) in greenhouses for 30 days in the first year and 60 days after bud flush in the second year. Other environmental conditions were controlled at optimal. After the first CO_2 exposure, net CO_2 assimilation (NA), stomatal conductance (g_s), intercellular to leaf surface CO_2 ratio (Ci/Ca) and transpiration rate (E) were increased by both CO_2 enrichments, but no provenance differences were found. In the second CO_2 exposure, NA and WUE significantly increased in all provenances at both CO_2 elevations. For the two southwest provenances, g_s were significantly decreased by 540 PPM, but not by the 720 PPM treatment. However, g_s of the two north-shore provenances did not respond to both CO_2 elevations. When measured at common CO_2 level, a 10% down regulation of NA was observed for the seedlings in the 720 PPM treatment, but no provenance differences were found. In the final harvest, the total, shoot, stem and root biomass were increased by CO_2 elevations, while leaf mass and biomass allocations were not. Most biomass components were increased by CO_2 elevations in the southwest provenances, but not in their north-shore counterparts. Biomass allocations were not significantly affected by CO_2 elevations. There were also no provenance differences in biomass allocations in response to elevated CO_2, while family differences only existed in stem mass ratio and leaf mass ratio. In conclusion, the two southwest provenances could perform better than the two north-shore ones in the elevated atmospheric CO_2 environment in future.

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CHAPTER ONE: LITERATURE REVIEW

Trembling aspen: A brief review

Trembling aspen (*Populus tremuloids* Michx.) is a clonal angiosperm and the most widespread tree species in North America (Burns and Honkola 1990). In Ontario, aspen regeneration is almost all of sucker origin. Seedlings can be seen only on severely disturbed areas with bare mineral soils and water table near the surface, such as heavily burned sites or road cuts (Heeny et al 1980). In spite of the dominance of its vegetative reproduction, trembling aspen is a species that has high intraspecific genetic variation (Cheliak and Dancik 1982; Mitton and Grant 1996).

Over its wide distribution range, climatic conditions vary greatly, especially minimum temperature and annual precipitation. Trembling aspen only occurs where annual precipitation exceeds evapotranspiration (Burns and Honkala 1990). It grows on many soil types and grows faster than other sympatric woody species. The typical soil types for trembling aspen in northwestern Ontario are medium/rich, dry soils, and it grows best on low to medium moisture, silt loam/clay (Heeny et al 1980; Buse and Bell 1992).

Trembling aspen is an important species both economically and ecologically. There is a great demand of its wood for lumber, pulp, veneer and the manufacture of various wafer boards (Heeny et al 1980). Aspen forests also provide natural habitats for wildlife and are an excellent indicator of high biodiversity (Kay 1997). Because of its short rotation, trembling aspen is being examined in various tree improvement programs around the world for use in intensive silvicultural systems.

Intraspecific variation in tree characteristics

Applied forest tree improvement programs were initiated and developed rapidly in the early 1950s (Zobel and Talbert 1984). Many tree improvement programs were then set up to maximize wood production at the shortest possible rotations at a reasonable cost by selecting additive heritable traits. To achieve this objective, appropriate tree characteristics must be selected, and broad genetic testing programs need to be conducted.

Tree improvers and breeders have examined the intraspecific variation of many characteristics of woody plants to find appropriate selection criteria. Currently, three groups of traits have been investigated: morphological/growth traits, biochemical/molecular traits and ecophysiological traits. Most studies on intraspecific variation are conducted on morphological/growth traits.

(1) Intraspecific variation in growth and morphological characteristics

Significant variations in root characteristics of *Populus balsamifera* L. have been reported at population and clonal level (Schnekenburger and Farmer 1989), and also in branch traits and height (Riemenschneider and Mcmahon 1993). Variation of *Populus tremuloides* Michx. in bud set and height has also been reported at clonal level (Thomas et al. 1997b). *Picea mariana* (Miller) B.S.P. showed clonal variation in shoot phenotypes (Johnsen et al 1996) and in height (Nienstaedt 1984). Genetic variation of *Pinus strobus* L. in branch numbers and bud set, bud burst (Li et al. 1997) and of *Picea Pungens* Engelm. in foliage colors and bud burst (Bongarten and Hanover 1986) are also significant at clonal level. Normally, such studies require 3 to 10 years, or even longer to complete because of the slow growth of trees.

(2) Intraspecific variation in biochemical and molecular traits

The use of biochemical/molecular traits for tree improvement purposes has been developed in recent years. The results are encouraging. For example, significant intraspecific variations of isozyme markers have been found among populations of trembling aspen (Hyun et al 1987; Lund et al 1992) and balsam poplar (Farmer et al 1988). RAPD (random amplified poly-DNA) tests also showed significant differences within and among populations, and even ramets of trembling aspen (Yeh et al 1995; Tuskan et al 1996; Stevens et al 1999). Wood extractives from trembling aspen also showed significant variation among different clones (Yanchuk et al 1988). However, some studies have showed that such variations are not correlated with growth (Farmer et al 1988; Chong et al 1994). Because of the complexity of gene controls, these biochemical/molecular traits are usually considered selectively neutral. They may not reflect intraspecific variations at the provenance level in characteristics that are important to the final yield and adaptation (Li 1995).

(3) Intraspecific variation in ecophysiological traits

Although the physiological traits of trees have been studied intensively, the use of physiological measurements to assist tree improvement programs is relatively new (Dunlap et al 1993; Thomas et al 1997a). Assessment of the heritability and genetic gain of physiological traits has largely been unexplored (Thomas et al 1997a). Since ecophysiological traits are inheritable and reflect the interactions between genotypes and the environment (Dang et al 1994), more research efforts should be made to explore the potential of using ecophysiological traits as selection criteria. Studies of genetic variation in ecophysiological traits may provide fundamental information on the mechanisms of adaptation, which can help us understand their functional

significance and their evolutionary heritage (Lambers et al 1998). Among ecophysiological traits, leaf gas exchange and biomass allocation are often investigated because of their importance to plant growth (Isebrands et al 1988; Reighard and Hanover 1990).

Net CO_2 assimilation is an important leaf gas exchange parameter. The maximum rate of net CO_2 uptake under saturated light can be used to predict shoot growth and indicate genetic variance (Ceulemans and Impens 1980). Intraspecific variations in net CO_2 assimilation have been reported for many woody species, e.g., *Malus* spp. (Mika and Antoszewski 1972), *Alnus rugosa* (Du Roi) Spreng. (Dang et al 1994), *Populus tremuloides* (Thomas et al 1997a) and *Pinus sylvestris* L. (Brunes et al 1980). Although there are more studies on the photosynthesis of *Populus* than any other tree genus (Isebrands et al 1988), the results vary significantly, e.g., the correlation between net assimilation rate and growth in poplar hybrids ranges from low to high (Gatherum et al 1967; Okafo and Hanover 1978; Ceulemans and Impens 1980; Briggs et al 1986). The reasons for the discrepancy vary; for example, environmental variability, inadequate or differing nitrogen supply, varying leaf age when leaf primordia were formed, and the difference in endogenous diurnal and seasonal cycles have been implicated (Tschaplinski and Blake 1989). Thomas et al (1997a) showed a positive correlation between net assimilation rate and growth of natural clones of trembling aspen under simulated field conditions in growth chambers. In addition to the rate of net assimilation, the total photosynthetic CO_2 uptake of a whole tree has also been positively correlated with biomass measurements (Isebrands et al 1988). This result suggests that measurement at either leaf or canopy level can provide important information on tree growth.

Other leaf gas exchange parameters, such as light compensation point (LCP), CO_2 compensation point, stomatal conductance to CO_2 (g_s), transpiration rate (E), and photosynthetic

water use efficiency (WUE), have also been used in assessing intraspecific variation of woody species. Some researchers report that WUE and boundary layer resistance to leaf gas exchange were significantly different among clones of *Populus* species (Ceulemans and Impens 1987), while others have reported significant variation in g_s and WUE among clones and populations (Thomas et al 1997a). However, some studies found no significant intraspecific difference in the dark respiration of *Populus* (Gatherum et al 1967; Okafo and Hanover 1978).

The allocation of photosynthetic assimilates or biomass, is another important ecophysiological trait that can affect the early development of seedling and wood quality of mature trees. Genetic variations at clone level in biomass allocation have been reported for poplar hybrids (Gatherum et al 1967; Tschaplinski and Blake 1989). Significantly different root-shoot ratios among clones and populations of trembling aspen have also been reported (Thomas et al 1997b). These studies suggest that seedlings with a high root/shoot ratio would show more vigorous juvenile growth and better field performance than seedlings with a low root /shoot ratio (Reighard and Hanover 1990).

Hogg (1999) has developed a carbon-based model to simulate responses of trembling aspen stands to climatic variation and insect defoliation. The model outputs of stem biomass were highly sensitive to parameters describing leaf longevity, insect defoliation intensity, photosynthetic and root respiration, and carbon allocation to growth *versus* storage. The results suggest that leaf gas exchange and biomass allocation should be good indicators of intraspecific variation in growth.

Seedling screening in genetic testing programs

Genetic testing is mandatory for any aggressive and successful tree improvement program (Zobel and Talbert 1984). It lays the foundation for genetic decisions involving the management of orchards and provides the information basis for advanced tree improvement efforts. Progeny testing is the best way to estimate the parental breeding values and to evaluate the genetic worth of selected parents. This test enables one to separate superior genotypes without the influence of environmental factors. Although such tests are effective, they generally require a long time to complete (e.g., 4-10 years), and the costs are very high. Seedling screening eliminates the need for rotation-length trials, which may be necessary for a progeny test and reduces the overall selection costs.

Most seedling screenings are based on morphological/growth traits, e.g., height, diameter, stem volume and root characteristics. Significant genetic variations of seedlings in these traits have been detected at provenance and clonal levels in many woody plants (Bongarten and Hanover 1986; Li et al 1997; Xu et al 1997). Studies on *Pinus pinaster* Ait. (Danjon 1994), *Picea mariana* (Miller) B.S.P. (Pharis et al 1991) and *Acer saccharum* Marshall (Schuler 1994) found that juvenile height was a good predicator of the performance of mature trees. A study on short leaf pine (*Pinus echinata*) (Tauer and McNew 1985) also showed that the height of a second year seedlings was a reliable predicator for the stem volume at the age of 10 years. Although there is a lack of information on the correlation between juvenile and mature trembling aspen trees, it is generally believed that it maintains high growth rate in its whole rotation. The early seedling screening of trembling aspen could be an additive benefit to current mature trees testing programs (Ledig 1974). However, although seedling screening is successful for morphological

and growth traits, it is unknown whether growth can be predicted from physiological traits in seedlings (Pharis et al 1991).

Ledig (1974) noted that for fast growing tree species, the combination of photosynthetic and respiratory rates together with parameters expressing the allocation of photosynthate between leaves, stem, and roots in seedlings, could be used to assess genetic differences in tree growth almost perfectly if seasonal growth patterns remain consistent from year to year. The results of several physiological studies have supported this viewpoint including studies on natural or hybrid poplar clones (Ceulemans and Impens 1980, 1987; Isebrands et al 1988; Hu et al 1997; Orlovic et al 1998) and *Picea mariana* provenances (Johnsen and Seiler 1996; Johnsen et al 1996). The studies on poplars were usually conducted on selected hybrid clones. Therefore, the need for quantitative genetic parameter estimates on both morphological and ecophysiological traits demands the testing of more genotypes and that testing be done on unselected native clones in addition to hybrids (Thomas et al. 1997a).

The heritabilities of ecophysiological traits in seedling screening couldn't be estimated accurately based only on controlled environmental experiments. The relationship between physiological performance and the final yield should be established (Thomas et al 1997a). Previous studies on poplar hybrids have shown both high and low correlations between the physiological traits of seedlings in greenhouse and field growth (Okafo and Hanover 1978; Ceulemans and Impens 1980, 1987; Briggs et al 1986; Tschaplinski and Blake 1989; Thomas et al 1997a). The low correlation was thought to result from the non-predictable, drastic changes of the environment conditions in the field, such as storms and extraordinary drought (Tschaplinski and Blake 1989; Thomas et al 1997a). The high correlation was observed in well-tended and

watered semi-field conditions (Cellemans and Impens 1987). More research is needed to evaluate this relationship.

Carbon dioxide elevation and tree improvement

(1) Global climate change and forests

Greenhouse gases are a direct cause for the current global climate change. Carbon dioxide (CO_2) is a major component of greenhouse gases. The global atmospheric CO_2 concentration has risen from the pre-industrial level of 280 PPM in 1750 to 367 PPM in 1999, and the increase is mostly caused by human activities. Furthermore, several General Circulation Models predict that the atmospheric CO_2 concentration will rise to 540 PPM to 940 PPM by 2100 (IPCC 2001).

Elevated CO_2 concentration has the potential to increase forest production by directly affecting tree physiology (McGuire and Joyce 1995). However, several other key environmental factors, which are important to tree growth, will also change simultaneously and rapidly (Aber et al 2001). For example, temperature is predicted to rise 1 to 5 ^{o}C globally due to greenhouse gases; precipitation generally will increase in North America but may decease in some regions; and nitrogen decomposition will intensify (Aber et al 2001). These changes further complicate the effects of elevated CO_2 concentration on forest productivity and intraspecific distribution (Aber et al 2001). At the same time, climate change can affect forests by altering the frequency, intensity, duration, and timing of natural disturbances, such as fire, drought, introduced species, insect and disease outbreaks, hurricanes, windstorms, ice storms, or landslides (Dale et al 2001).

In conclusion, elevated CO_2 concentration and the subsequent environmental changes will likely change the forest ecosystem in the future. We cannot predict with certainty, how

forests will respond to the global change because we cannot carry out multisite, multifunctional experiments required to do so (Aber 2001). However, we still can use the results of single-factor experiments to provide some indications of how individual tree species will respond under simulated conditions.

(2) Effects of elevated CO_2 on woody plants

The direct effects of CO_2 elevation on woody plants will be the significant changes in physiological processes, and subsequent changes in phenology and growth. The most consistent effect is an increase in the rate of photosynthetic carboxylation and a reduction in photorespiration, leading to increased rates of net photosynthesis and tree growth, at least in the short term (Aber et al 2001). In the review of 500 papers on tree responses to high CO_2, Curtis and Wang (1998) found that woody plant total biomass and net assimilation increased from 16% to 52% depending on other limiting environmental factors, but there were no significant shifts of biomass allocation in response to doubled CO_2 concentration.

Long term CO_2 exposure studies suggest that down-regulation of photosynthesis occurred over time (Lambers et al 1998). One recent study on a perennial herb *Plantago lanceolata* showed a 21% down-regulation of net assimilation rate, when measured at common internal CO_2 pressure (Klus et al 2001). The downward acclimation of net assimilation is also shown in *Picea sitchensis* (Borg) Carr. (Centritto and Jarvis 1999). However, photosynthetic down-regulation does not occur in all plants; for example, no photosynthetic down-regulation was found for *Liquidambar styraciflua* L. (sweetgum) in field trials when exposed to high CO_2 concentration (Herrick and Thomas 2001). Net assimilation of trembling aspen also does not demonstrate downward photosynthetic acclimation to high CO_2 (Curtis et al 2000). The decline in

photosynthesis in response to elevated CO_2 found in those studies could also have been caused by other factors, such as water or nutrient stress imposed on pot-grown seedlings where root growth is limited (Curtis and Wang 1998).

The responses of physiological variables can vary with species and/or genotypes. For example, *Pseudotsuga menziesii* (Douglas fir) showed no stomatal conductance changes in response to elevated CO_2, and the transpiration rate of Douglas fir was not affected by elevated CO_2 (Apple et al 2000). For trembling aspen, there are reports that some genotypes had no stomatal conductance reductions in response to CO_2 enrichment (Wang et al 2000), but others had significant reduction in stomatal conductance (Radoglou and Javis 1990; Wang et al 2000). Leaf dark respiration rates of nine deciduous species (Amthor 20000) and root respiration rates of *Fagus sylvatica* L. (European beech) (Leveranz et al 1999) did not decrease significantly in response to elevated CO_2. Because of the variability of growth and photosynthetic responses, woody plants do display species-specific response mechanisms to CO_2 elevation (Hattenschwiler 2001).

The interactions between CO_2 and other environmental factors, such as nutrients, water and light, are likely high, affecting trees responses to CO_2 elevation (Aber et al 2001). For example, plant growth is generally stimulated by CO_2 elevation under optimal conditions, but this stimulation changes with the magnitude of the biomass enhancement ratios (plant total biomass in high CO_2 divided by total biomass in current CO_2 level) under sub- or supra-optimal environmental conditions (Pooter and Perez 2001). In their review, large variability of interactions between CO_2 and the environment were apparent between experiments. Low nutrient availability or low atmospheric temperatures reduced the growth response to elevated CO_2, while reduced irradiance or high salinity caused mixed responses in biomass production (Pooter and

Perez 2001). In another review (Kerstiens 2001), enhancement in biomass was significantly higher in shade tolerant species than in non-shade tolerant species.

(3) Impact of CO_2 enrichment on tree improvement programs

Climate changes will probably result in changes to the natural distribution of forest tree species. Elevated CO_2 concentration, acting as a selection pressure, may also affect genetic structure of the boreal forest (Colombo et al 1998). These changes certainly will affect current and future tree improvement programs. To evaluate seed sources for future use, it is important to understand how local seed sources will respond, physiologically and morphologically to CO_2 elevation.

Different species have shown various responses to changing environmental conditions (Kerstiens 2001; Hattenschwiler 2001; Pooter and Perez 2001). In the review of CO_2 enrichment experiments, Curtis and Wang (1998) report woody plants have demonstrated interspecific variation in photosynthetic responses to elevated CO_2, and 16% to 36% increases in net assimilation are predicted in response to the doubling of atmospheric CO_2 concentration. However, genetic variation in traits associated with adaptation to local conditions exists not only at the species level, but also at the provenance, family and individual level (Thompson 1998).

The intraspecific variation of the responses of woody species to elevated CO_2 is less known than the interspecific variation, and needs further investigation. Several studies reported different clonal responses in stomatal conductance (Radoglou and Javis 1990; Wang et al 2000) and biomass allocation of poplar hybrids (Lindroth et al 2001), and net CO_2 assimilation of trembling aspen (Kalina and Ceulemans 1997; Wang et al 2000). At population level, *Picea mariana* (Miller) B.S.P. demonstrated weak variation (Johnsen and Seiler 1996), and *Pinus*

ponderosa showed evident among-provenance variability (Houpis et al 1999). Furthermore, *Plantago lanceolata* expresseed strong variation at family level in response to CO_2 enrichment (Klus 2001 et al 2001).

However, some species showed no significant provenance differences in physiological and morphological traits to elevated CO_2, e.g., *Picea sitchensis* (Centritto and Jarvis 1999) and *Fagus* (Leveranz et al 1999). This result may be because of the small number of provenances used and the close geographic origins of the provenances; e.g., CO_2-family interaction was significant in an experiment with 18 families in one experiment, while CO_2-population interaction was not significant with only two populations (Klus et al 2001). Alternatively, it may be because woody species have different intraspecific responses to elevated CO_2, which means the intraspecific differentiation may be suppressed in some species but prompted in others by high atmospheric CO_2 concentration (Thompson 1998). Further studies on intraspecific variation to elevated CO_2 are required to provide guidelines for future tree improvement programs.

CHAPTER TWO: GENETIC VARIATION IN PHYSIOLOGICAL AND GROWTH PERFORMANCE OF TREMBLING ASPEN AT AMBIENT CO_2

INTRODUCTION

Tree improvement combines silvicultural and tree breeding skills to grow the most valuable forest products as quickly and inexpensively as possible. The first step for any intensive tree improvement program is to understand quantitative genetic variability within the desired species, and then to select superior individuals or families to provide the genetic source for reforestation (Zobel and Talbert 1984). Because of the long rotation of forest tree crops, there is a need to shorten the selection time, which will reduce costs and promote intensive selection. Seedling screening, as an indirect selection method, may provide important information for current selection programs for the current climate and for future climate conditions.

Morphological/growth traits are economically important. Intraspecific variations in height and breast height diameter at the provenance level are reported in many woody species, such as *Populus balsamifera* L. (Riemenschneider and Mcmahon 1993), *Populus tremuloides* Michx. (Thomas et al 1997b), *Picea mariana* (Miller) B.S.P. (Nienstaedt 1984; Johnsen et al 1996), *Pinus strobus* L. (Li et al 1997) and other species (Carter 1996). Recent studies report high genetic correlations between rotation age and the rate of juvenile growth in several species, e.g. *Acer saccharum* Marshall (Schuler 1994), *Picea mariana* (Miller) B.S.P. (Pharis et al 1991), *Pinus pinaster* Ait. (Danjon 1994) and *Pinus echinata* (Tauer and McNew 1985). These results suggest the seedling screening using morphological/growth traits may be applicable for tree improvement programs.

Ecophysiological traits are inheritable physiological traits that reflect the acclimation and adaptation of plant species to their environment, and studying these traits can help us understand their functional significance and their evolutionary heritage (Lambers et al 1998). Although the physiological traits of trees have been studied intensively, the use of physiological measurements to assist the determination of superior trees is relatively new (Dunlap et al 1993; Thomas et al 1997a). Among ecophysiological traits, leaf gas exchange and biomass partition are often chosen because of their importance to growth (Isebrands et al 1988; Reighard and Hanover 1990). Genetic variation in leaf photosynthetic traits has been reported in many woody species, e.g., *Malus* spp (Mika and Antoszewski 1972), *Alnus rugosa* (Du Roi) Spreng. (Dang et al 1994), *Populus tremuloides* Michx. (Thomas et al 1997a) and *Pinus sylvestris* L. (Brunes et al 1980). Genetic variation in the partition of photosynthate has also been reported in poplar hybrids (Gatherum et al 1967; Tschaplinski and Blake 1989).

Early seedling screening based on ecophysiological traits is beneficial to tree improvement programs (Ledig 1974). Leaf photosynthetic traits and partition of biomass in seedlings could be used to reveal genetic differences in tree growth (Ledig 1974). Photosynthetic studies of poplar hybrids (Ceulemans and Impens 1980, 1983; Hu et al 1997; Orlovic et al 1998) and *Picea mariana* (Miller) B.S.P. (Johnsen and Seiler 1996; Johnsen et al 1996) support this opinion, and suggest that the use of ecophysiological traits in tree improvement programs should be encouraged in future research.

Trembling aspen (*Populus tremuloides* Michx.) is the most widely distributed tree species in North America (Burns and Honkala 1990), and may have the highest intraspecific genetic variation among all woody species (Mitton and Grant 1996). It occupies sites with a wide range of climatic conditions and soil types. It is a pioneer species at or on disturbed sites. Aspen stands

also provide natural habitats for various wildlife (Kay 1997). Because of its economic importance, many tree improvement programs have been set up to select superior families (Li 1995). However, most studies are restricted to hybrids because of their better performance and higher genetic gain. For tree improvement purposes, more genotypes, especially natural clones that have broader genetic background, should be tested in addition to hybrids (Thomas et al 1997a).

The goals of this study were to investigate genetic variation in ecophysiological and morphological traits of trembling aspen in northwestern Ontario and to determine the adaptive nature of these traits. Hypotheses tested were: 1) that above traits are genetically controlled and represent adaptations to local environments and 2) that genetic differences exist between both families and provenances.

MATERIAL AND METHODS

Plant materials

Seeds of twenty-seven trembling aspen provenances (populations) were collected in 1996 from northwestern Ontario (Figure 2.1) and stored at –5 °C. Four provenances were selected for this study. Two provenances, P1 (48°29′35″ N, 90°48′57″ W) and P3 (48°27′45″ N, 90°35′02″ W), were from southwest of Thunder Bay. The other two provenances, P25 (48°57′13″ N, 87°58′13″ W) and P26 (49°03′38″ N, 87°58′28″ W) were from the north-shore of Lake Superior. Three open pollinated families were selected from each provenance.

Climate data for the provenances were derived from a digital climate model for Ontario (Mackey et al 1996). The mean daily maximum temperatures in the growing season (June to August) were 22.87°C and 22.57°C, respectively, for P1 and P3, and 21.27°C and 21.17°C,

respectively, for P25 and P26. The mean daily minimum temperatures in the growing season (June to August) were 9.67°C and 9.07°C, respectively, for P1 and P3, and 9.30°C and 8.83°C, respectively, for P25 and P26. The mean monthly precipitation in the growing season were 95.47mm and 96.87mm, , and 85.60mm and 89.03mm, respectively, for P25 and P26. The soils in both areas were dry, shallow-moderately deep coarse sand (Elkie et al 2000).

Seeds were sown in May 2001 in plastic germination trays in the Lakehead University Greenhouse following commercial growing procedures as to water and fertilizer (Hackleman et al 2000). Twenty-five days after germination, 90 healthy seedlings per family were transplanted into styroblocks (45 cavities/block; 340ml/cavity). After another 50 days, 27 uniform trees per family were transplanted again to 6-inch pots before the start of the experiment.

Experimental design

The experiment was a completely randomized design with single tree plots and carried out in two phases in greenhouse. The first phase took place in the Lakehead University Main Greenhouse from May to August, 2001 and consisted of three blocks isolated on separate benches. At that time all of the seedlings were taken from one bench (one third of the total) and moved to the smaller Research Greenhouse and randomly subdivided into 3 blocks located on separate benches to begin the second experimental phase which continued to April, 2002. The other two blocks from the Main Greenhouse were transferred to separate greenhouses for a CO2 enrichment experiment (See chapter 3 for details). The experimental design was the same for both phases consisting of 3 blocks, each containing 4 provenances and 3 families nested within provenances. However, the number of seedlings per family was reduced from 9 to 3 in the Phase 2. Although two greenhouses were used for the two phases, the greenhouse environmental

conditions were similar and thus should not affect the design of the experiment. This decision is consistent with most greenhouse/common-garden growth experiments.

Environmental conditions promoting rapid growth were maintained throughout Phase 1 and Phase 2, in which the bud set and dormancy were excluded. During growing periods, the greenhouses were well ventilated, and day/night temperatures were set at around 24/12 °C. Relative humidity was between 50% and 80%. The photoperiod was maintained at 16-hour and the natural light was supplemented by high-pressure sodium lamps on cloudy days, early mornings and late evenings. The light intensity at canopy level was 270 to 320 μmol photons m^{-2} s^{-1}. The growing medium was a peat-vermiculite mixture (50/50, v/v). Seedlings were fertilized once a week using water solution of Nitrogen by Plantprod® 41-11-8 (100 PPM) and watered up to twice a day to keep soil moist.

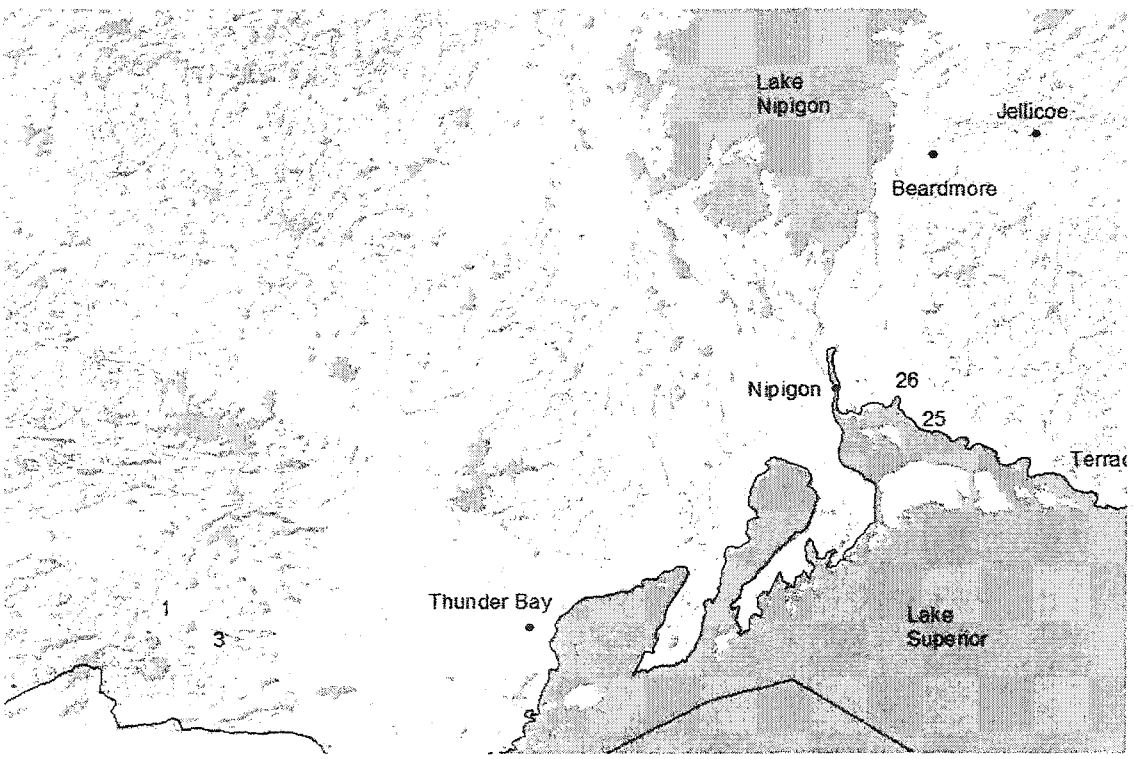

Figure 2.1 Seed sources of trembling aspen.

Leaf gas exchange measurement

At the end of Phase 1, (August 2001)[1], 100 days after germination, six of the nine seedlings per family were randomly selected from each block for the measurement of leaf gas exchange. The gas exchange was measured on the first fully expanded leaf from the top based on observations of relative leaf size (Thomas et al 1997); this was the leaf six in this study. The instantaneous light-saturated leaf CO_2 exchange rate was measured using a PP-system CIRAS-1 gas exchange system and a Parkinson broad-leaf chamber with automatic environmental control (PP-System, Haverhill, MA, USA). The measurements were taken at 22 ± 0.1°C air temperature, 360 ± 10 PPM CO_2 and 800 μmol m^{-2} s^{-1} photosynthetic active radiation. The measurement sequence was randomized within and among blocks to avoid systematic errors (Lee and Rawlings 1982; Dang et al 1994). In addition, the measurements were taken from 9:00 am to 4:30 pm to eliminate diurnal errors based on preliminary tests (Wang et al 2000). Net CO_2 assimilation (NA), stomatal conductance (g_s), leaf transpiration rate (E), and the internal to ambient CO_2 concentration ratio (Ci/Ca) were calculated according to von Caremmer and Farquhar (1981). Photosynthetic water use efficiency (WUE) was caulated as NA/E.

Thirty days after the start of Phase 2, in October 2001 (130 days after germination), two of three seedlings per family were randomly selected from each block for gas exchange measurements. After the measurements, environmental conditions in greenhouses were gradually set to outside conditions. The dormancy was introduced for all the seedlings in this process. After four months of dormancy, growth of all seedlings was stimulated by exposing them to 16-hour photoperiod and 25/15°C day/night temperatures. Sixty days after the buds flushed at the

[1] Although the concept of phase was used to better describe the sequence of the experiment, the months of the measurement (e.g., August 01, October 01 and April 02) were used instead in both chapter 2 and 3 for clear interpretation of different data sets.

conclusion of the second experimental phase (April 2002), two of the three seedlings per family in each block were again randomly selected for measurement of leaf gas exchange, which were same as described in August 2001 measurements.

Growth and biomass measurement

In August and October 2001, the height and root collar diameter (RCD) were measured on the seedlings used for gas exchange measurements. Height and RCD of seedlings were not measured in April 2002 because seedling heights had exceeded greenhouse space which may have complicated morphological data at measurement time.

After completion of the measurements in April 2002, all the seedlings in the three blocks were harvested and dried at 80°C for 48 hours to determine dry weight of foliage, stem and root. Total biomass, Leaf mass ratio (LMR = foliage mass to total mass ratio), stem mass ratio (SMR = stem mass to total mass ratio), root mass ratio (RMR = root mass to total mass ratio) and root/shoot ratio (RSR = root mass to shoot mass ratio) were calculated. The leaf area of the single leaf that was used for gas exchange measurements on a sample tree was measured using Winfolia® leaf analysis system (Regent Instruments Inc., Quebec City, Canada). Those leaf samples were dried and weighted separately from other leaves to determine the specific leaf area (SLA). The whole plant leaf area per seedling (LA) was then determined from the SLA and the total foliage dry mass.

Data analysis

For all three measurements, the data were analyzed using the following linear model of analysis of variance (ANOVA):

$$Y_{ijkl} = \mu + B_i + P_j + BP_{ij} + F_{(j)k} + BF_{i(j)k} + \varepsilon_{(ijk)l}$$

Where Y_{ijkl} = the measured response of the *l*th seedling replicate in the *i*th block from the *k*th family nested in the *j*th provenance

μ = overall mean

B_i = the random effect of the *i*th block (i = 1, 2, 3)

P_j = the random effect of the *j*th provenance (j = 1, 2, 3, 4)

$F_{(j)k}$ = the random effect of the *k*th family within the *j*th provenance (k = 1, 2, 3)

BP_{ij} = the interaction of the *i*th block and the *j*th provenance

$BF_{i(j)k}$ = the interaction of the *i*th block and the *k*th family within the *j*th provenance

$\varepsilon_{(ijk)l}$ = the random error due to *l*th seedling from the *k*th family within the *j*th provenance in the *i*th block (l = 1, 2, 3, 4, 5, 6-August 2001 measurements; l = 1, 2-October 2001 and April 2002 measurements)

Because the samples in each measurement were different from other measurements, repeat measure test of ANOVA was not adopted. ANOVA was conducted separately for each measurement. The expected mean squares for each term in the linear model were given in Table 2.1.

Table 2.1 EMS table for ANOVA of growth experiment.

Sources of Variation	df	EMS
B_i	2	$\sigma^2 + N\sigma^2_{BF} + NF\sigma^2_{BP} + NFP\sigma^2_B$
P_j	3	$\sigma^2 + N\sigma^2_{BF} + NB\sigma^2_F + NF\sigma^2_{BP} + NFB\sigma^2_P$
BP_{ij}	6	$\sigma^2 + N\sigma^2_{BF} + NF\sigma^2_{BP}$
$F_{(j)k}$	8	$\sigma^2 + N\sigma^2_{BF} + NB\sigma^2_F$
$BF_{i(j)k}$	16	$\sigma^2 + N\sigma^2_{BF}$
$\varepsilon_{(ijk)l}$	36	σ^2
Total	71	

Note: Where in Table 1, P, F, B and N are respectively, the number of provenances (4), the number of families per provenance (3), the number of blocks (3) and the number of seedlings per experiment unit (2 or 6).

All the variables were considered as random in the analysis. All the tests were conducted using the PROC GLM, RANDOM/TEST option of SAS/STAT® 8.2 statistical software (SAS Inc. 1989). Variance components were calculated by hand using the appropriate mean square values and the corresponding mean square coefficients generated by SAS (Table 2.1). Negative variance components were assumed to be zero (Thomas et al 1997b).

Narrow sense heritabilities (h^2) were calculated on both family and single tree bases. The following equations were used for heritability estimation based on expected mean square coefficients:

$$h^2_f = \sigma^2_F / (\sigma^2_F + \sigma^2_{BF}/B + \sigma^2/NB);$$

$$h^2_s = 4*\sigma^2_F / (\sigma^2_F + \sigma^2_{BF} + \sigma^2).$$

Where σ^2_F is the variance due to family within provenance, and σ^2_{BF} is the variance due to family and block interaction, and σ^2 is variance due to error. B and N are, respectively, the number of blocks (3) and the number of seedlings per experiment unit (2 or 6).

Simple correlation analysis was used to examine the relationship between leaf gas exchange and both growth and biomass traits. Genetic correlation analyses were also conducted to examine genetic relationships between all the variables. The calculation of genetic correlation between two variables was done using the following formula from Stonecypher (1992)

$$\text{Genetic correlation} = \frac{(V_{(1+2)} - V_1 - V_2)/2}{\sqrt{V_1 * V_2}}.$$

Where V_1 is the family variance of the first variable, V_2 is the family variance of the second variable and $V_{(1+2)}$ is the family variance of the corresponding sum of the first and second variables.

To investigate whether variation expressed among provenances had adaptive significance, provenance mean values for gas exchange, growth and biomass variables were regressed against monthly climate data obtained for each provenance using a digital climate model (Mackay et al 1996). The climate variables used in the regression are mean monthly maximum and minimum temperatures, and mean monthly precipitation (36 variables total).

RESULTS

Genetic variation in gas exchange

There were no significant ($p > 0.05$) provenance effects on net CO_2 assimilation (NA), photosynthetic water use efficiency (WUE) and intercellular to leaf surface CO_2 concentration ratio (Ci/Ca). However, there were larger provenance components than family components of NA and Ci/Ca in August 2001 and NA in April 2002 measurements. There were significant differences between provenances ($p < 0.05$) in stomatal conductance (g_s) and transpiration rate (E) in August 2001 measurements (Table 2.2).

There was a nearly two-fold increase in overall NA in April 2002 in comparison to the first year (Figure 2.2a). WUE was generally higher in August 2001 and April 2002 measurements than in October 2001 (Figure 2.2b). g_s and E, on the other hand, were generally lower in August measurement than the two subsequent measurements (Figure 2.2c and 2.2e), particularly in provenance P3 and P26. Ci/Ca ratio was generally higher in August and October 2001 measurements than in April 2002 (Figure 2.2d). The provenance component for NA was erratic across the three measurements, and explained only 9% of the total variance in April 2002 measurement. The provenance contributed none or very little to the variation in WUE and Ci/Ca. However, provenance explained around 23% of variance in g_s and E in August 2001, but had become low and variable in October 2001 and April 2002 (Table 2.2).

Family effects were significant ($p < 0.05$) for all gas exchange variables in August 2001, ranging from 13% to 30% of the total variation. Family was only significant for g_s in October 2001 but still ranged from 4% to 23% of the total variation. By April 2002, the family component of variation had dropped to zero for all gas exchange variables (Table 2.2). Block and family interactions were significant for NA in all three measurements and for WUE and Ci/Ca in October 2001 measurement (Table 2.2). The family variances were 12.7% and 17.7% of the total for NA, but were about 30% and 6% for WUE and Ci/Ca, respectively, for both the August and October measurements in 2001. However, the variance explained by family was low but stable for g_s and E (Table 2.2). There were large amount of variance explained by Block * Family interaction for nearly all gas exchange variables in October 2001 and April 2002 measurements, except g_s in October 2001 and WUE and Ci/Ca in April 2002 (Table 2.2). The family and single tree heritabilities of gas exchange variables were high and variable in 2001 measurements, but became zero in April 2002 measurements (Table 2.2).

Table 2.2 Source of variation, degree of freedom (Df), p-values, percent variance (%VAR) explained and family/single tree heritabilities (h^2_f/h^2_s) for net CO_2 assimilation (NA), water use efficiency (WUE), stomatal conductance (g_s) and intercellular to leaf surface CO_2 ratio (Ci/Ca) and transpiration rate (E) for August 2001, October 2001 and April 2002.

NA

August 01				October 01				April 02			
Source	Df	p-value	%VAR	Source	Df	p-value	%VAR	Source	Df	p-value	%VAR
B	2	0.90	0.0	B	2	0.40	0.2	B	2	0.75	0
P	3	0.10	14.1	P	3	0.47	0.3	P	3	0.28	9.1
B*P	6	0.58	0.0	B*P	6	0.66	0.0	B*P	6	0.06	23.5
F(P)	8	0.04	12.7	F(P)	8	0.15	17.7	F(P)	8	0.46	0.3
B*F(P)	16	0.02	8.4	B*F(P)	16	<0.0001	52.4	B*F(P)	16	0.03	24.6
Error	180		64.8	Error	36		29.3	Error	36		42.5
h^2_f/h^2_s		0.66/0.59		h^2_f/h^2_s		0.44 /0.72		h^2_f		0.02/0.01	

WUE

August 01				October 01				April 02			
Source	Df	p-value	%VAR	Source	Df	p-value	%VAR	Source	Df	p-value	%VAR
B	2	0.51	0.0	B	2	0.76	0.0	B	2	0.23	2.2
P	3	0.64	0.0	P	3	0.80	0.0	P	3	0.75	0.0
B*P	6	0.59	0.0	B*P	6	0.31	6.4	B*P	6	0.62	0.0
F(P)	8	0.0002	29.1	F(P)	8	0.36	4.1	F(P)	8	0.84	0.0
B*F(P)	16	0.37	1.3	B*F(P)	16	0.01	37.2	B*F(P)	16	0.64	0.0
Error	180		69.6	Error	36		52.2	Error	36		97.8
h^2_f/h^2_s		0.87/1.17		h^2_f/h^2_s		0.16/0.18		h^2_f/h^2_s		0.00/0.00	

g_s

August 01				October 01				April 02			
Source	Df	p-value	%VAR	Source	Df	p-value	%VAR	Source	Df	p-value	%VAR
B	2	0.34	0.1	B	2	0.12	4.7	B	2	0.41	0.2
P	3	0.04	23.2	P	3	0.30	5.8	P	3	0.41	2.4
B*P	6	0.87	0.0	B*P	6	0.60	0.0	B*P	6	0.46	0.0
F(P)	8	0.004	15.3	F(P)	8	0.03	22.9	F(P)	8	0.48	0.0
B*F(P)	16	0.36	2.1	B*F(P)	16	0.33	5.6	B*F(P)	16	0.08	27.1
Error	180		59.3	Error	36		61.1	Error	36		70.3
h^2_f/h^2_s		0.79/0.80		h^2_f/h^2_s		0.66/0.99		h^2_f/h^2_s		0.00/0.00	

Ci/Ca

August 01				October 01				April 02			
Source	Df	p-value	%VAR	Source	Df	p-value	%VAR	Source	Df	p-value	%VAR
B	2	0.60	0.0	B	2	0.71	0.0	B	2	0.30	1.2
P	3	0.30	4.9	P	3	0.84	0.0	P	3	1.00	2.2
B*P	6	0.54	0.0	B*P	6	0.30	6.5	B*P	6	0.75	0.0
F(P)	8	0.0008	30.1	F(P)	8	0.26	8.0	F(P)	8	0.88	0.0
B*F(P)	16	0.23	3.2	B*F(P)	16	0.04	29.7	B*F(P)	16	0.50	0.0
Error	180		61.8	Error	36		55.7	Error	36		96.6
h^2_f/h^2_s		0.87/1.26		h^2_f/h^2_s		0.29/0.34		h^2_f/h^2_s		0.00/0.00	

E

August 01				October 01				April 02			
Source	Df	p-value	%VAR	Source	Df	p-value	%VAR	Source	Df	p-value	%VAR
B	2	0.56	0.0	B	2	0.10	6.9	B	2	0.21	5.0
P	3	0.04	23.8	P	3	0.17	13.2	P	3	0.37	3.5
B*P	6	0.81	0.0	B*P	6	0.59	0.0	B*P	6	0.45	0.5
F(P)	8	0.005	18.5	F(P)	8	0.12	13.3	F(P)	8	0.50	0.0
B*F(P)	16	0.15	3.6	B*F(P)	16	0.08	18.2	B*F(P)	16	0.10	23.1
Error	180		54.2	Error	36		48.4	Error	36		67.9
h^2_f/h^2_s		0.81/0.98		h^2_f/h^2_s		0.48/0.67		h^2_f/h^2_s		0.00/0.00	

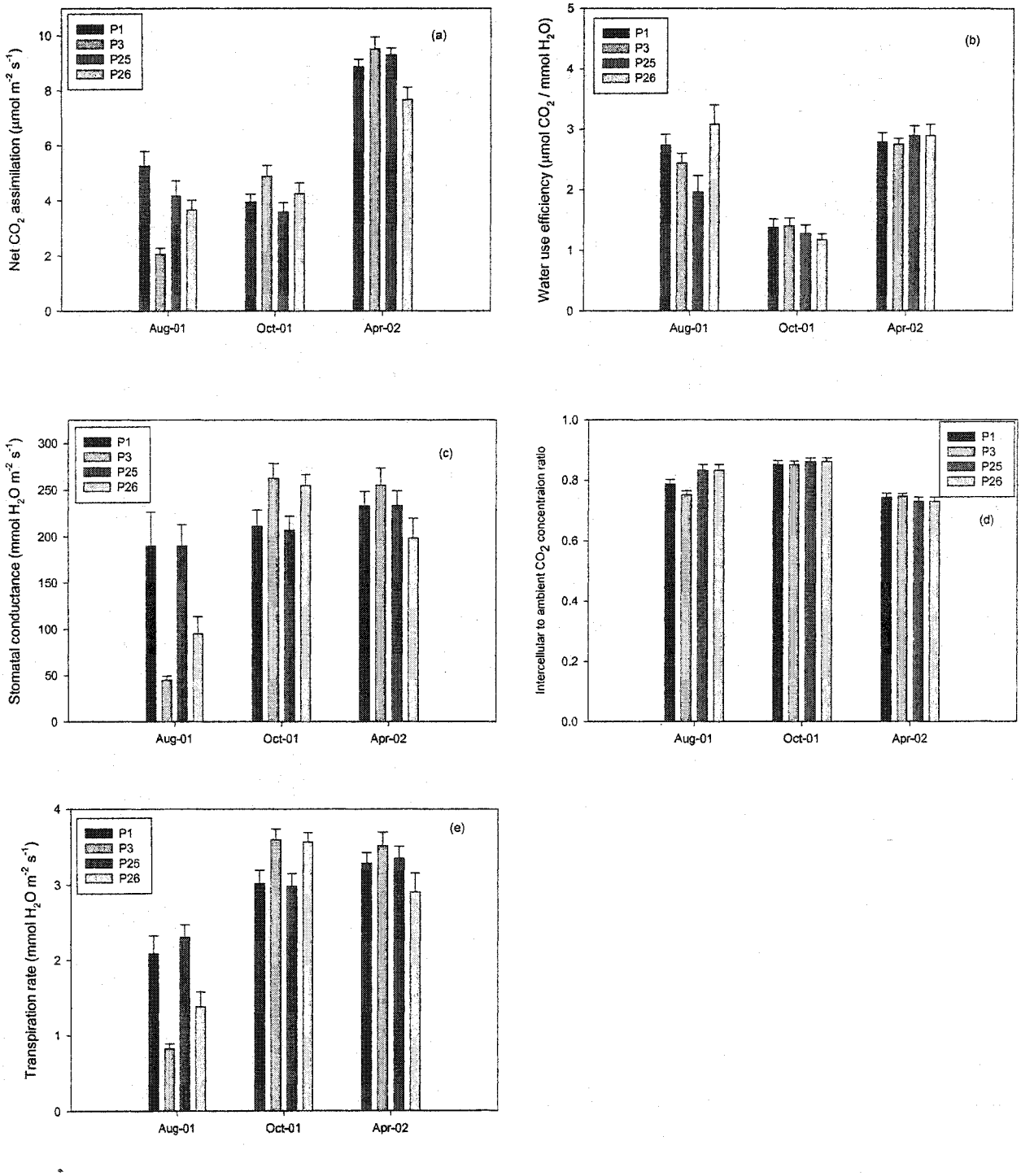

Figure 2.2 NA (a), WUE (b), g_s (c), Ci/Ca (d) and E (e) (Mean ± 1 SE) of trembling aspen seedlings from four provenances in northwestern Ontario in three measurements.

Note: 216 seedlings were measured in August 2001, and 72 seedlings in October 2001 and April 2002.

Genetic variation in growth and biomass

There was a significant ($p < 0.05$) provenance effect on height growth in August 2001 after 100 days of growth, but there were no significant ($p > 0.05$, Table 2.3and Figure 2.3a) provenance effects in the October 2001 measurement when growth had slowed. There were no significant differences between provenances in root collar diameter (RCD) in either August or October 2001 measurements. No significant ($p > 0.05$, Table 2.4 and Figure 2.4b) provenance effects were found in biomass components, allocation or whole plant leaf area (LA) in the final harvest in April 2002.

In spite of low significant level (Table 2.3), there were very high components of growth variations attributable to provenance. In first year (2001) growth, provenance explained 47% and 42% of the variance in RCD and height respectively (Table 2.3). Provenance also accounted for 13.7% and 18.7% of the variance in total and stem biomass in the final harvest (Table 2.4a). However, provenance only explained about 2% and 10% of the variance in leaf and root biomass. P25 had lowest ranking in RCD and height in 2001 (Figure 2.3a and 2.3b) and in biomass components in April 2002 (Figure 2.3c). Furthermore, provenance almost did not contribute to the variation (0.0-4.1%) in biomass allocations (Table 2.4b).

Family effects were significant ($p < 0.05$) for RCD and height in August 2001, but not in October 2001 (Table 3). There were significant family effects on the total, shoot and leaf biomass (Table 4a), and on the leaf mass ratio (LMR), root mass ratio (RMR), root to shoot ratio (RSR) and LA (Table 4b). No significant family effects were found for stem, root biomass and stem mass ratio (SMR) (Table 4).

Table 2.3 Source of variation, degree of freedom (Df), p-values, percent variance (%VAR) explained and family and single tree heritabilities (h^2_f/h^2_s) of four provenances of trembling aspen for root collar diameter (RCD) and height measured in August 2001(a) and October 2001 (b)

a) August 2001

		RCD		Height	
Source	Df	p-value	%VAR	p-value	%VAR
B	2	0.27	0.3	0.52	0.0
P	3	0.06	47.0	0.03	42.2
B*P	6	0.01	1.3	0.09	1.2
F(P)	8	<0.0001	37.3	<0.0001	32.7
B*F(P)	16	0.99	0.0	0.79	0.0
Error	180		26.3		23.9
h^2_f/h^2_s		0.89/2.34		0.89/2.31	

b) October 2001

		RCD		Height	
Source	Df	p-value	%VAR	p-value	%VAR
B	2	0.06	6.8	0.20	2.1
P	3	0.77	24.5	0.24	31.2
B*P	6	0.73	0.0	0.85	0.0
F(P)	8	0.18	8.5	0.48	0.0
B*F(P)	16	0.15	12.8	0.01	31.2
Error	36		47.4		35.5
h^2_f/h^2_s		0.41/0.49		0.00/0.00	

Table 2.4 Source of variation, degree of freedom (Df), p-values, percent variance (%VAR) explained and family and single tree heritabilities (h^2_f/h^2_s) of four provenances of trembling aspen for biomass components (a) and for biomass allocation variables and whole plant leaf area at harvest in April 2002.

a) Biomass

		Total		Shoot		Stem		Leaf		Root	
Source	Df	p-value	%VAR	p-value	%VAR	p-value	%VAR	p-value	%VAR	p-value	%VAR
B	2	0.37	0.4	0.31	0.9	0.07	6.7	0.79	0.0	0.60	0.0
P	3	0.08	13.7	0.11	11.4	0.07	18.7	0.38	1.6	0.18	10.0
B*P	6	0.06	6.1	0.08	4.9	0.58	0.0	0.03	8.0	0.07	11.8
F(P)	8	0.01	11.0	0.01	12.5	0.27	3.7	0.001	20.0	0.15	6.6
B*F(P)	16	0.99	0.0	0.99	0.0	0.66	0.0	0.99	0.0	0.79	0.0
Error	36		68.9		70.3		70.9		70.5		71.6
h^2_f/h^2_s		0.49/0.55		0.52/0.60		0.24/0.20		0.63/0.88		0.36/0.34	

b) Biomass allocation

		SMR		LMR		RMR		RSR		LA	
Source	Df	p-value	%VAR	p-value	%VAR	p-value	%VAR	p-value	%VAR	p-value	%VAR
B	2	0.22	6.2	0.27	4.1	0.62	0.0	0.56	0.0	0.10	5.9
P	3	0.87	0.0	0.92	0.0	0.93	0.0	0.93	0.0	0.28	4.1
B*P	6	0.21	9.2	0.15	9.9	0.18	8.0	0.17	7.3	0.20	3.7
F(P)	8	0.18	10.8	0.03	26.5	0.05	14.7	0.05	14.7	0.01	15.6
B*F(P)	16	0.07	21.3	0.09	19.8	0.76	0.0	0.74	0.0	0.94	0
Error	36		52.5		39.7		78.3		78.0		70.7
h^2_f/h^2_s		0.41/0.51		0.67/1.23		0.53/0.63		0.53/0.63		0.57/0.72	

Note: SMR is stem mass to total mass ratio; LMR is leaf mass to total mass ratio; RMR is root mass to total mass ratio: RSR is root mass to total mass ratio; LA is whole plant leaf area (m^2).

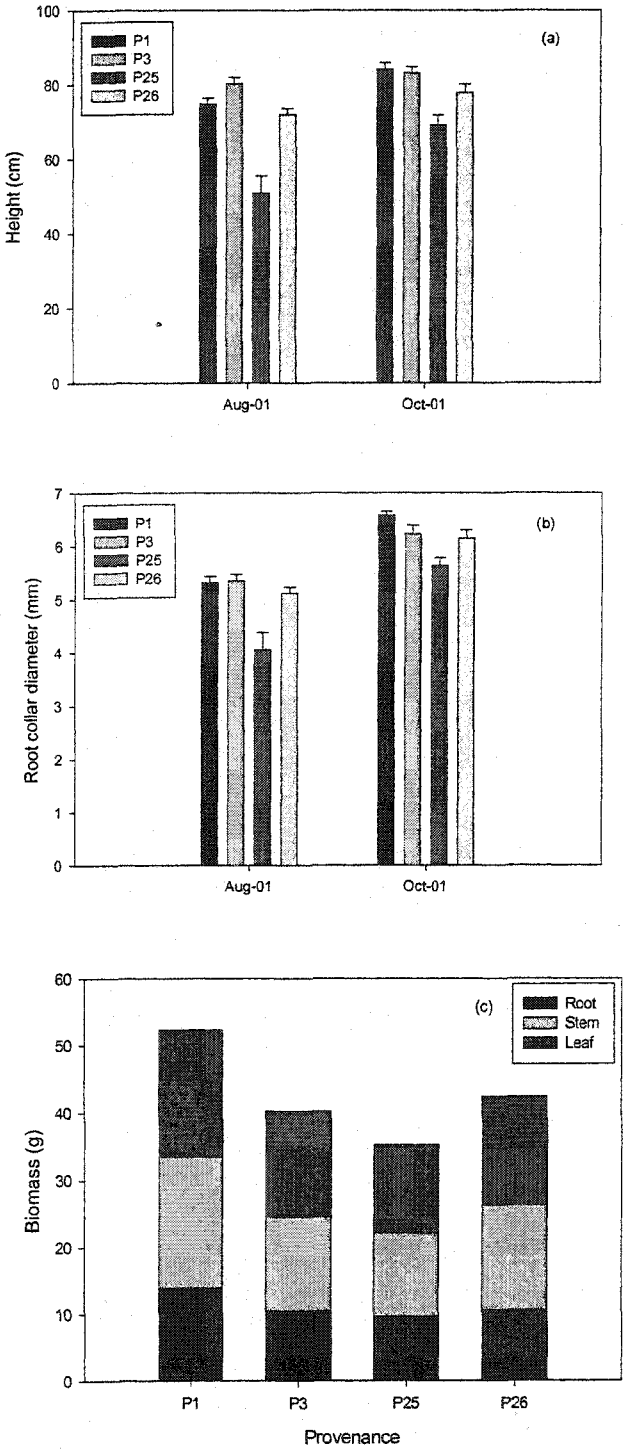

Figure 2.3 Height (a), RCD (b) and biomass components (c) (mean ± 1 SE) of trembling aspen seedlings from four provenances in northwestern Ontario growing under greenhouse conditions.

Note: Sample size was 216 seedlings for August 2001 measurement and 72 for October 2001 measurement and biomass measurements.

Although family explained a large proportion of variance in RCD and height (37% and 33% respectively) in August 2001 (Table 2.3a), family contributed little to the stem biomass (3.7%). Provenance explained more variance of stem mass than family (18.7% for provenance and 3.7% for family), but family explained more variance of leaf mass (1.6% for provenance and 20.0% for family). Nearly equal proportions of variance in shoot mass (11.4% and 12.5% respectively) were explained by provenance and family (Table 2.4a). Provenance and family explained only a small proportion of variance in root mass (10.0% and 6.6% respectively). In contrast to biomass components, the variation in biomass allocations was completely accounted for by family: 11% to 27% of variance in allocation variables, and 16% of variance in LA (Table 2.4b).

Family heritability estimates were high for RCD and height in August 2001, but declined in October 2001 because of the lower family variance (Table 2.3). For single tree heritability, the estimates for RCD and height were out of normal range (> 1) in August 2001, but were lowered for RCD and disappeared for height in October 2001 measurements (Table 2.3). For biomass components, both family and single tree estimates were high for total, shoot and leaf mass, but relatively low for stem and root mass (Table 2.4a). For biomass allocations, heritability estimates were relatively high too (Table 2.4b).

Pearson correlation, genetic correlation and regression with climate

There were consistent but weak correlations between leaf gas exchange and growth or biomass on a single tree basis (Table 2.5). For example, WUE was positively correlated with height in August ($r=0.12$, $p=0.08$) and October 2001 ($r=0.25$, $p=0.03$), and with total biomass in April 2002 ($r=0.19$, $p=0.10$). Ci/Ca were negatively correlated with height in August ($r=-0.13$,

Table 2.5 Pearson (lower half) and genetic (upper half) correlations of gas exchange and growth traits for four provenances of trembling aspen seedlings from northwestern Ontario in three measurements.

Time									
		A	WUE	E	Ci/Ca	g_s	RCD	Height	
August 2001	A		0.27	0.21	0.02	0.48			
	WUE	0.393***		0.19		0.04		0.03	
	E	0.653***	-0.312***		0.63	0.86	0.05	0.13	
	Ci/Ca	-0.184***	-0.948***	0.478***		0.31		0.01	
	g_s	0.593***	-0.274***	0.936***	0.452***				
	RCD	0.008	0.094	-0.132**	-0.074	0.006		0.96	
	Height	-0.026	0.116*	-0.178***	-0.128*	-0.053	0.880***		
		A	WUE	E	Ci/Ca	g_s	RCD	Height	
October 2001	A			0.48		0.18			
	WUE	0.709***							
	E	0.330**	-0.373***		0.65	0.06			
	Ci/Ca	-0.654***	-0.985***	0.423***					
	g_s	0.290**	-0.355***	0.955***	0.438***				
	RCD	0.320***	0.244**	0.030	-0.242**	-0.007			
	Height	0.388***	0.252**	0.132	-0.241**	0.107	0.636***		
		A	WUE	E	Ci/Ca	g_s	SLA	Total	LA
April 2002	A								
	WUE	-0.009							
	E	0.604***	-0.778***						
	Ci_Ca	0.070	-0.986***	0.798***					
	g_s	0.546***	-0.773***	0.976***	0.814***				
	SLA	-0.038	-0.168	0.103	0.154	0.087			
	Total	-0.052	0.192*	-0.181	-0.196*	-0.187	-0.412***		0.82
	LA	0.001	0.074	-0.077	-0.071	-0.092	0.099	0.714***	

Note: 1. There were 216 seedlings in August 2001, and 72 seedlings in October 2001 and April 2002.
2. Asterisks indicate significance level: * = $p \leq 0.10$, ** = $p \leq 0.05$, *** = $p < 0.01$. The meanings of abbreviations see Tables 2, 3 and 4.

p=0.06) and October 2001 (r=-0.24, p=0.04), and with total biomass in April 2002 (r=-0.20, p=0.10). NA was only correlated with height in October 2001 (r=0.39, p=0.001). However, there was a positive correlation between LA and total biomass (r=0.71, p=0.001) and negative correlation between SLA and total biomass (r=0.41, p<0.001) in the April 2002.

Genetic correlations were generally lower than Pearson correlation values, and many could not be calculated due to low family components of variance (Table 2.2, 2.3 and 2.4). There were also weak genetic correlations between gas exchange and height (Table 2.5). There was a weak genetic correlation between WUE and height (r_g= 0.03) in August 2001, but such a relationship couldn't be found in October 2001. Moreover, no genetic correlation was found between WUE and biomass in April 2002. However, there was a genetic correlation between E and height in August 2001 (r_g=0.13).

There were also genetic correlations between gas exchange variables (Table 5). For example, there were genetic correlations between NA and E in August (r_g=0.21) and in October 2001 (r_g=0.48), and between NA and g_s in August (r_g=0.48) and October 2001 (r_g=0.18). High genetic correlation between E and Ci/Ca were also found in August (r_g=0.63) and in October 2001 (r_g=0.65). No genetic correlations (between 0 and 1) were found between other gas exchange variables in any of three measurements.

Although there were only four provenances in the simple regression, the analyses showed that many gas exchange/growth variables could be predicted by monthly climate variables (Table 2.6 and Figure 2.4). For example, there were six gas exchange/growth variables predicted by January mean minimum temperature (Table 2.6). The late-season (August to October) monthly mean minimum temperatures predicted many gas exchange/growth variables for the four provenances ($p<0.05$). Mid-season (April to July) monthly mean maximum temperatures and mean precipitation ($p<0.05$), and winter monthly climate variables also predicted gas exchange and growth variables ($p<0.05$, Table 2.6). However, no biomass and biomass allocation variables could be predicted by monthly mean climate variables.

Table 2.6 The R-squares of simple regressions between monthly climate variables and August 01, October 01 and April 02 measurement data (gas exchange and growth) for four provenances of trembling aspen from northwestern Ontario. Only significant R-squares were presented here.

	August 2001					October 2001						April 2002		
	E	Ci/Ca	g_s	Height	RCD	A	WUE	E	Ci/Ca	g_s	Height	WUE	Ci/Ca	SLA
JanMinT	0.81*	0.87*		0.82*		0.80*		0.80*		0.82*				
AprMinT									0.84*					
MayMinT														0.84*
JunMinT									0.80*					
JulMinT														0.98***
AugMinT	0.92**		0.83*	0.80*		0.98***				0.80*				
SepMinT	0.84*			0.88*		0.94**								
OctMinT	0.92**		0.83*	0.80*		0.98***				0.80*				
NovMinT				0.83*							0.87*	0.90**	0.82*	
DecMinT				0.96**	0.89**						0.88*			
JanMaxT				0.97***	0.91**	0.82*					0.82*			
FebMaxT														0.86*
MarMaxT							0.83*		0.88*					
AprMaxT							0.85*		0.89**			0.82*	0.89*	
MayMaxT							0.80*		0.84*			0.91**	0.93**	
JunMaxT							0.80*		0.83*			0.89**	0.92**	
JulMaxT							0.82*		0.86*			0.87*	0.91**	
AugMaxT							0.84*		0.88*			0.80*	0.86*	
SepMaxT									0.80*					0.83*
OctMaxT														0.93**
NovMaxT				0.92**							0.86*			
DecMaxT				0.96**	0.88*						0.87*			
JanMnP							0.86*		0.90**			0.85*	0.91*	
FebMnP								0.88*		0.82*				
MarMnP	0.80*		0.89**					0.96**		0.94**				
AprMnP				0.81*							0.91**	0.90**	0.84*	
MayMnP											0.87*	0.94**	0.89**	
JunMnP											0.91**	0.90**	0.85*	
JulMnP											0.85*	0.95**	0.91**	
AugMnP		0.91**		0.80*										
SepMnP		0.83*						0.84*						
OctMnP							0.91**		0.94**			0.84*	0.91**	
NovMnP							0.89**		0.93**			0.86*	0.92**	
DecMnP							0.89**		0.93**			0.85*	0.91**	

Note: 1. Asterisks indicate significance level: * = $p \leq 0.10$, ** = $p \leq 0.05$, *** = $p < 0.01$.
2. The meanings of gas exchange and growth abbreviations see previous tables.
3. JanMinT means mean Janurary minimum temperature; JanMaxT means mean January maximum temperature; JanMnP means mean January precipitation; so were true for other climate abbrevations.

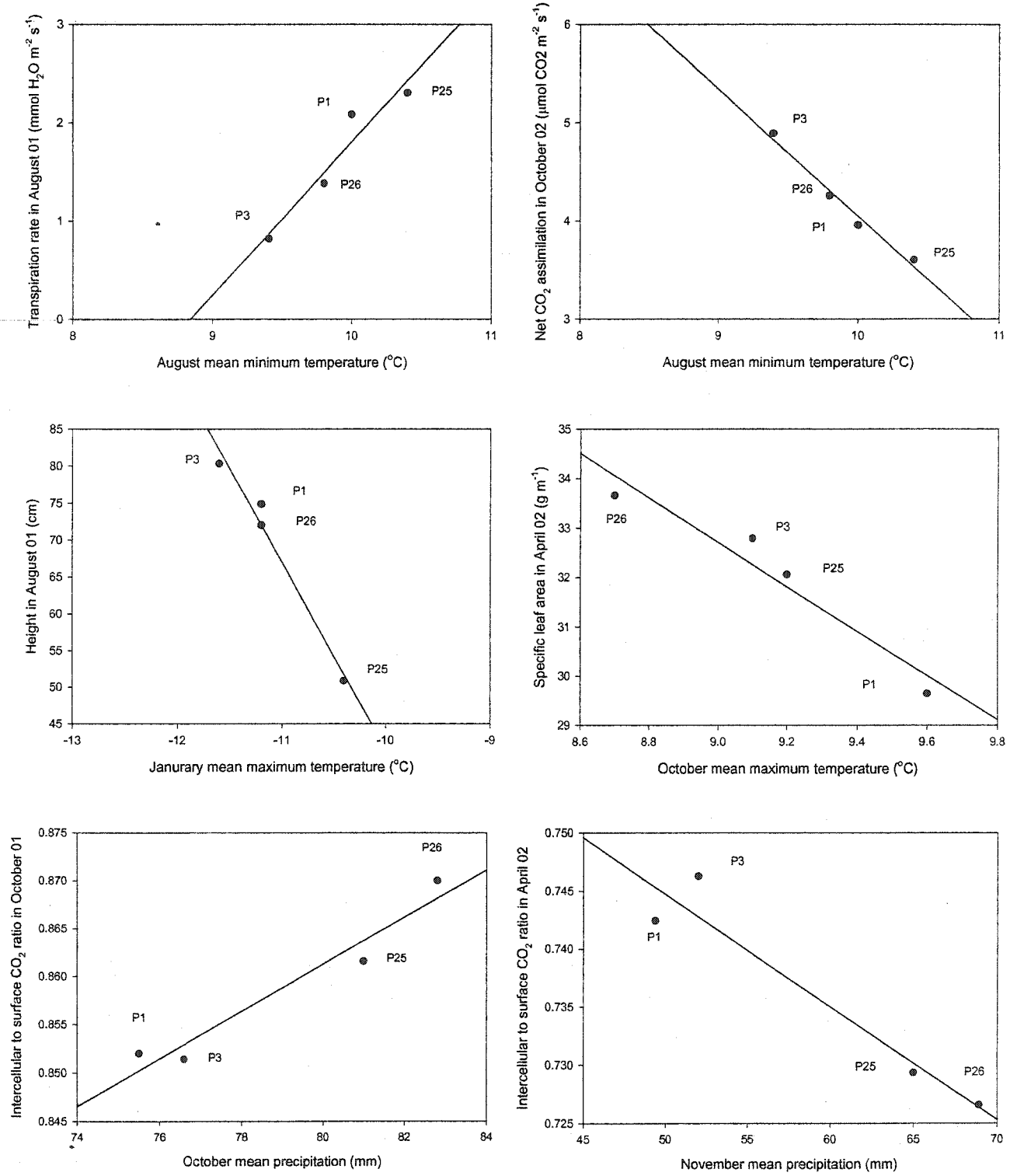

Figure 2.4 Examples of simple regressions between climate variables and gas exchange/growth variables.

DISCUSSION

High clonal variation has been reported for morphological, growth and physiological traits in trembling aspen (Cheliak and Dancik 1982; Jelinski and Cheliak 1992; Thomas et al 1997a, b). In this study, substantial variation at the family level was found in leaf gas exchange in four native provenances of trembling aspen from northwestern Ontario (Table 2.2). However, variance explained by family in leaf gas exchange (0-29%) in this study is less than the clonal variation (0-59%) in a larger scale seedling-screening test in Alberta (Thomas et al 1997a).

There were significant provenance variations in g_s and E in August 2001 measurements. Because all seedlings were under extreme high temperatures in summer time, evaporation demand was high in the greenhouse. The variation under such conditions may be related to the local climates of the provenances. For example, E was highly correlated to the mean August minimum temperature.

Variations at provenance and family levels were reduced in October 2001 with the exception of family variations in NA and g_s. Because the measurements were taken after bud set, the October 2001 gas exchange data represented late-growing season gas exchange. Kubiske et al (1998) reported that differences between trembling aspen genotypes faded inside open top chambers late in the growing season because of high temperature inside the chamber late in the season. Therefore, the reduction of provenance variation in leaf gas exchange and growth traits (RCD and height) in October 2001 could have resulted from the artificial growth environment.

The net CO_2 assimilation (NA) values in this study were similar to the values reported for container or root box grown poplar seedlings (Ceulemans et al 1986; Kubiske et al 1997; Thomas et al 1997a), but lower than measurements on field grown seedlings or mature trees (Okafo and Hanover 1978; Isebrands et al 1988; Wang et al 2000). However, the overall NA

values of April 2002 were twice as high as those of year 2001. Furthermore, the NA values of April 2002 were approaching reported values in the field (Thomas et al 1997a). This finding may indicate the existence of seasonal or developmental changes of trembling aspen seedlings or both. Environmental restrictions could be another factor for the low NA in this study. Before the measurements of seedlings were taken in August 2001, they had been in the relatively small cavities of styroblock (345ml) and under relatively high temperatures and high evaporation demand for 50 days. Although the seedlings were later transplanted again into larger pots (6-litre) for 25 days, the restriction of the previous environmental conditions might have already resulted in a lower photosynthetic capacity. However, the environmental conditions were much more favorable during leaf expansions in 2002 in comparison to the previous year.

Both the family and single tree heritability estimates for leaf gas exchange were relatively high but variable in the first year. The estimates are generally higher than those of broad sense heritabilities of one-year-old trembling aspen in Alberta (Thomas et al 1997a). However, heritability estimates of leaf gas exchange dropped to zero for April 2002. This extreme decrease was unexpected compared to the results of other field or growth chamber studies on trembling aspen (Isebrands et al 1988; Thomas et al 1997a). This result might suggest provenance and family differences were present only under certain environmental conditions, e.g., intense evaporation and high temperature in summer 2001. Alternatively, provenance and family differences in leaf gas exchange may have seasonal patterns, i.e., differences are present in the summer but not in spring. Furthermore, significant block by family interactions in all gas exchange variables in October 2001 and April 2002 measurements showed the high sensitivity of genotypes to surrounding environment (Table 2.2). Because some of the seedlings in some block were high enough to exceed supplementary lamps in October 2001 and April 2002, the uneven

light conditions in artificial photoperiod could explain the block by family interactions. Finally, the result also suggests that due to the experimental conditions, the variance initially expressed among families was subsequently manifested by block and family interaction variance (Table 2.2).

The provenances demonstrated substantial variation in growth (RCD and height), total and stem biomass (Table 2.3 and 2.4a). Family variations were only significant in RCD and height in August 2001, and in total, shoot and leaf biomass of final harvest in April 2002. These findings were consistent with the study of Thomas et al (1997b) on native trembling aspen. Family and single tree heritability estimates for growth and biomass components in this study, most of which were close to 1, were much higher than reported broad sense heritabilities (Thomas et al 1997b). These high values may reflect enhanced genetic expression under controlled environment in the earliest stage of this experiment, because a large portion of the environmental 'noises' were minimized under controlled environment. However, the heritability of stem mass was close (0.24/0.20) to other morphological estimates in trembling aspen clones (Thomas et al 1997b).

Compared to growth and biomass, variances of biomass allocations were explained more by family than by provenance, resulting in high heritabilities for biomass allocations. This result corresponded to high broad sense heritabilities in biomass allocation reported for poplar clones in growth chamber and field studies (Tschaplinski and Blake 1989; Thomas et al 1997b). The high block by family interaction in SMR and LMR detected in this study showed the high sensitivity of genotypes to the environment regarding biomass allocation as well as leaf gas exchange (Table 2.4b).

In this study, NA did not show any significant correlation with growth or biomass on single tree basis except in October 2001. Studies of quantitative genetics of *Populus* on physiological traits, such as leaf gas exchange and biomass allocations, have typically concentrated on hybrid clones (Gatherum et al 1967; Ceulemans and Impens 1980; Orlovic et al 1998), or failed to consider family structure (Ceulemans and Impens 1987; Wang et al 2000). If the quantitative genetic information about physiological traits is to be used in a tree improvement program, the relationship between carbon fixation and growth must be established (Thomas et al 1997a). However, the leaf gas exchange of trembling aspen seedlings is very sensitive to environmental conditions, such as light, temperature and water (Okafo and Hanover 1978; Reghard and Hanover 1990; Thomas et al 1997a). It is not unusual for different studies to report contradictory relationships between NA and growth (Ceulemans and Impens 1980, 1987; Briggs et al 1986; Hu et al 1997; Orlovic et al 1998). Thomas et al (1997a) reported positive correlations between NA and height and root dry weight of trembling aspen in a growth chamber experiment. In another study, NA had significant but very weak (r^2=0.02, p=0.0001) relationship with total biomass in three-year-old trembling aspen clones (Wang et al 2000). The genetic correlations between gas exchange and total biomass in this study were also significant but low (Table 5).

WUE had consistent positive, but low correlation with height and total biomass in all three measurements on a single tree basis. This finding also corresponds to the previously reported weak correlations between gas exchange and growth or biomass variables found in Wang et al (2000). However, leaf traits, such as LA and SLA, showed strong correlations (r=0.71 and r=0.41) with total biomass on a single tree basis. There was also a high genetic correlation between LA and total biomass (r=0.82), but not for SLA. The above findings suggest that low

correlation coefficients of leaf gas exchange and biomass are not very useful in seedling screening, but leaf traits could play an important role in tree improvement programs.

This study showed that many climates variables were good predictors of provenance performances (Table 2.6). For example, NA in October 2001 had a very high regression coefficient (0:98) with August and October mean minimum temperatures, and height in August 2001 had a regression coefficient of 0.97 with January mean maximum temperature (Figure 2.4). Such tight relationships between climate and provenance performance under a common environment suggest that these four provenances are adapted to the local climates. This finding indicates that local seed sources of aspen should be used for future improvement programs in this area.

In conclusion, native trembling aspen exhibited genetic variation in leaf gas exchange, biomass components and biomass. The use of physiological traits in tree improvement programs is still debatable (Thomas et al 1997a). This study indicates such uses may not be very effective for trembling aspen seed sources in northwestern Ontario because of the lack of a good relationship between physiological traits and growth. This greenhouse study provides us important genetic information on local trembling aspen seed sources. However, more tests are needed to establish relationships between controlled environment and field conditions in physiological or growth performance (Thomas et al 1997a, b).

CHAPTER THREE: EFFECTS OF CO_2 ENRICHMENT ON GAS EXCHANGE AND BIOMASS

INTRODUCTION

Global climate is changing mainly due to increases in greenhouse gases emitted by human activities (IPCC 2001). Carbon dioxide (CO_2) is the major greenhouse gas driving the climate change. The global atmospheric CO_2 concentration has risen from the pre-industrial level of approximately 280 PPM in the late 18th century to 355 PPM in 1991 and continues to increase at the rate of 1.8 PPM per year (Watson et al 1992). Based on the outputs of General Circulation Models, the atmospheric CO_2 concentration will reach between 540 PPM and 900 PPM by year 2100 (IPCC 2001).

The elevated CO_2 concentration has the potential to increase forest productivity by directly affecting tree physiology (McGuire and Joyce 1995). The most consistent effect is an increase in the rate of carboxylation by the photosynthetic system and a reduction in photorespiration leading to increased rates of net photosynthesis and tree growth, at least in the short term (Aber et al 2001). Curtis and Wang (1998) found that the total biomass and net assimilation of woody plants increased from 16% to 52% in response to elevated CO_2 depending on other limiting environmental factors, with no significant shifts in biomass allocation.

Photosynthetic down-regulation is the negative feedback to increasing CO_2. Long term CO_2 exposure studies suggest that down-regulation of photosynthesis occurs over time (Lambers et al 1998; Centritto and Jarvis 1999; Klus et al 2001). However, photosynthetic down-regulation does not occur in all species (Curtis et al 2000; Herrick and Thomas 2001). Significant changes in other physiological processes, phenology and growth are also observed in plants grown under elevated CO_2 (Aber et al 2001).

Elevated CO_2 concentration can also be viewed as an additional selection pressure that may affect the genetic structure of the boreal forest (Colombo et al 1998). The genetic structure of forests will change if the frequencies of current genotypes change in response to the environment change. In the context of changing environment, understanding genotype and environment interactions is critical for understanding the possible evolutionary responses to changes in the environment (Klus et al 2001). Although the intraspecific variation of woody plants in photosynthetic responses to elevated CO_2 has been documented (Curtis and Wang 1998), such variation is still not well understood. Several studies have shown clonal differences in the response of stomatal conductance to CO_2 enrichment by poplar hybrids (Radoglou and Javis 1990), in biomass allocation (Lindroth et al 2001) and net assimilation by *Populus tremuloides* Michx. (Kalina and Ceulemans 1997; Wang et al 2000). At the provenance level, *Picea mariana* (Miller) B.S.P. has demonstrated weak genetic variation (Johnsen and Seiler 1996) while *Pinus ponderosa* has shown evident genetic variation in net assimilation rate in response to elevated CO_2 (Houpis et al 1999). Furthermore, a few studies have determined the basic organization of inheritable variation within a species, i.e., population and family levels (Johnsen and Seiler 1996; Klus et al 2001). The partitioning of variation among families and populations plays a critical role in determining the magnitude of responses to natural selection (Klus et al 2001). For example, *Plantago lanceolata* has expressed strong photosynthetic variation in response to elevated CO_2 at both provenance and family level (Klus et al 2001).

Some species do not show significant provenance differences in physiological and morphological traits in response to elevated CO_2, e.g., *Picea sitchensis* (Bong) Carr. (Centritto and Jarvis 1999) and *Fagus sylvatica* (Leveranz et al 1999). The different responses between different species are not well understood (Thompson 1998). The complexity of interspecific and

intraspecific variations in responses of woody plants to a changing environment has been a challenge to tree improvement programs. Predictions based on experiments under the current climate may not be appropriate for the future climate. Given the long rotation of trees, the risk of applying such predictions in tree improvement programs can be huge. Therefore, the genetic variation of trees in response to elevated CO_2 needs to be investigated to provide guidelines for current and future tree improvement programs.

Trembling aspen (*Populus tremuloides* Michx.) is an important tree species in the boreal forest both ecologically and economically. The purpose of this study was to investigate the genetic variation of trembling aspen in growth and ecophysiological responses to CO_2 enrichment under greenhouse conditions.

MATERIAL AND METHODS

Plant materials

Seed sources and seedlings were as described in Chapter 2.

Experimental design

This experiment was a nested split-plot design (Hicks 1993). The CO_2 concentrations in three greenhouses were set at: ambient, 540 PPM (1.5 * ambient) and 720 PPM (2 * ambient). There were three blocks nested in each greenhouse. There were four provenances in each block, three families per provenance, and three seedlings per family. The locations of seedlings in each block were randomized within the block. The actual CO_2 concentrations in the three greenhouses were: 381 ± 12, 537 ± 33 and 727 ± 64 PPM. Other environmental conditions were similar in all

three greenhouses. All the environmental conditions in all three greenhouses were monitored and controlled using the Argus® controlling system (Vancouver, Canada).

When the Phase 1 of the experiment started on August 25th 2001, the seedlings were 100 days old with mature leaves and were starting to set buds. After 30 days of treatments, foliar gas exchanges were measured. At the beginning of October 2001, the seedlings were transplanted from 5-inch pots into 6-litre pots. The environmental conditions in the greenhouses were then gradually changed to winter storage conditions (–4 °C). CO_2 enrichments were stopped during the storage.

The Phase 2 of the experiment was started on February 23rd, 2002. When the CO_2 treatment was started again, the environmental conditions were changed to growing conditions. Because the height of the greenhouses became limiting to seedling growth, the CO_2 treatments only lasted for 60 days.

Leaf gas exchange measurement

At the end of the Phase 1 (October 2001), two seedlings per family in each block were randomly selected for leaf gas exchange measurements. The measurements of gas exchange were taken on the sixth leaf from the top using a PP-system CIRAS-1 gas exchange system and a Parkinson broad-leaf chamber with automatic environmental control (PP-System, Haverhill, MA, USA). The measurements were taken at the corresponding growth CO_2 levels. Other environmental conditions in the leaf chamber were 22 ± 0.1°C air temperature, 800 μmol m^{-2} s^{-1} PAR and 50-80% RH. The sequence of measurements was randomized within and among blocks and greenhouses to avoid systematic errors (Lee and Rawlings 1982; Dang et al 1994). All the measurements were taken from 9:00 am to 4:30 pm to eliminate diurnal errors (Wang et al 2000).

Net CO_2 assimilation (NA), stomatal conductance (g_s), leaf transpiration rate (E), and the intercellular to leaf surface CO_2 concentration ratio (Ci/Ca) were calculated according to *von* Caremmer and Farquhar (1981). Photosynthetic water use efficiency (WUE) was defined as NA/E.

Sixty days after the start of the Phase 2 (April 2002), two seedlings from every family within each block in each CO_2 treatment were randomly selected for gas exchange measurements as described previously. In order to determine the photosynthetic down-regulation, each seedling in the CO_2 enrichment treatments was measured at the corresponding growth CO_2 and ambient CO_2 concentration. The seedlings in the ambient CO_2 treatment were measured only at the ambient CO_2 concentration.

Biomass measurements

After the gas exchange measurement in Phase 2 (April 2002), all the seedlings were harvested and dried at 80°C for 48 hours to determine the dry mass of foliage, stem and roots. The total biomass, Leaf mass ratio (LMR = leaf mass to total mass), stem mass ratio (SMR = stem mass to total mass), root mass ratio (RMR = root mass to total mass) and root/shoot ratio (RSR = root mass to shoot mass) were calculated. The leaf area of the leaf, of which gas exchange measurement was taken on each sample tree, was measured using Winfolia® leaf analysis software (Regent Instruments Inc., Quebec City, Quebec, Canada). The leaf samples were dried and weighed separately from other leaves to determine the specific leaf area (SLA). The whole plant leaf area (LA) was calculated from SLA and the total foliage dry weight.

Data analysis

The gas exchange and biomass data were analyzed using combined ANOVA with the following linear model where provenances were grouped:

$$Y_{ijklmn} = \mu + C_i + B_{(i)j} + \delta_{(ij)} + P_k + CP_{ik} + BP_{(i)jk} + F_{(k)l} + CF_{i(k)l} + BF_{(ik)jl} + \varepsilon_{(ijkl)m}$$

Y_{ijklm} = the measured response of the *m*th replicate in the *j*th block within the *i*th CO_2 treatment which from *l*th family in the *k*th provenance.

μ = overall mean

C_i = the fixed effect of the *i*th CO_2 treatment ($i = 1, 2, 3$)

$B_{(i)j}$ = the random effect of the *j*th block within the *i*th CO_2 treatment ($j = 1, 2, 3$)

$\delta_{(ij)}$ = whole plot restriction error

P_k = the random effect of the *k*th provenance ($k = 1, 2, 3, 4$)

$F_{(k)l}$ = the random effect of the *l*th family in the *k*th provenance ($l = 1, 2, 3$)

CP_{ik} = the interaction of the *i*th CO_2 treatment and the *k*th provenance

$CF_{i(k)l}$ = the interaction of the *i*th CO_2 treatment and the *l*th family in the *k*th provenance

$BP_{(i)jk}$ = the interaction of the *j*th block within the *i*th CO_2 treatment and the *k*th provenance

$BF_{(ik)jl}$ = the interaction of the *j*th block within the *i*th CO_2 treatment and the *l*th family within the *k*th provenance

$E_{(ijkl)m}$ = the random error due to the m*th* seedling in the *j*th block within the *i*th CO_2 treatment which from the *l*th family in the *k*th provenance ($m = 1, 2$).

Table 3.1 EMS table for ANOVA of CO_2 enrichment experiment.

Source of Variation	df	EMS
C_I	2	$\sigma^2 + FPB\,\sigma^2_\delta + FPC\,\sigma^2_B + FPBC\,\Phi(C)$
$B_{(i)j}$	6	$\sigma^2 + FPB\,\sigma^2_\delta + FPC\,\sigma^2_B$
δ	0	$\sigma^2 + FPB\,\sigma^2_\delta$
P_k	3	$\sigma^2 + N\,\sigma^2_{BF} + NB\,\sigma^2_{CF} + NBC\,\sigma^2_F + NF\,\sigma^2_{BP} + NBF\,\sigma^2_{CP} + NBFC\,\sigma^2_P$
CP_{ik}	6	$\sigma^2 + N\,\sigma^2_{BF} + NB\,\sigma^2_{CF} + NF\,\sigma^2_{BP} + NBF\,\sigma^2_{CP}$
$BP_{(i)jk}$	18	$\sigma^2 + N\,\sigma^2_{BF} + NF\,\sigma^2_{BP}$
$F_{(k)l}$	8	$\sigma^2 + N\,\sigma^2_{BF} + NB\,\sigma^2_{CF} + NBC\,\sigma^2_F$
$CF_{i(k)l}$	16	$\sigma^2 + N\,\sigma^2_{BF} + NB\,\sigma^2_{CF}$
$BF_{(ik)jl}$	48	$\sigma^2 + N\,\sigma^2_{BF}$
$\varepsilon_{(ijkl)m}$	108	σ^2
Total	215	

Note: Where in the table, C, P, F, B and N are respectively, the number of CO_2 treatments (3), the number of provenances (4), the number of families per provenance (3), the number of blocks (3) and the number of seedlings per experiment unit (2).

The expected mean squares for each term in the model were given in Table 3.1. The effect of CO_2 was considered to be fixed. The effects of block, provenance and family were assumed random. The CO_2 effect was tested against block as a split-plot test. All other variables were tested using PROC GLM, RANDOM/TEST option by SAS/STAT® 8.2 statistical software (SAS Inc. 1989). The biomass allocation data did not follow a normal distribution, and thus were subjected to log transformation. The results of ANOVA were transformed back to original unit for better interpretation of the data. Simple correlation was used to describe relationships between leaf gas exchange and biomass at different CO_2 levels. Simple regression was also used to describe relationships between gas exchange and monthly climate variables.

Because there were no F-tests for the interaction between CO_2 and provenance in combined ANOVA, an additional ANOVA model was conducted for each provenance, with only CO_2 as fixed factor, to examine whether a particular provenance was affected by CO_2 elevations. The Student-Newman-Keuls range tests were adopted for *post-hoc* multiple comparisons for both ANOVA models.

RESULTS

Gas exchange in Phase 1 (October 2001)

After 30 days of exposure to CO_2 treatments, all gas exchange variables except photosynthetic water use efficiency (WUE) were significantly increased by CO_2 elevations when tested by combined ANOVA (Table 3.2). However, the provenance effect was significant at $p<0.1$ for g_s and E. There were no significant provenance or family effects ($p > 0.05$) (Table 3.2). There were significant CO_2 - family interactions in WUE and intercellular to surface CO_2 ratio (Ci/Ca), but not in net CO_2 assimilation (NA), stomatal conductance (g_s) or transpiration rate (E) (Table 3.2). Block by family interactions were also significant for NA, WUE, and E ($p<0.05$) and Ci/Ca ($p<0.01$).

NA of seedlings grown at 540 and 720 PPM CO_2 were 48% and 55%, respectively, higher than that of seedlings grown at ambient CO_2 ($p < 0.05$, Table 3.2 and Figure 3.1a). No general significant difference was found between 540 and 720 PPM in overall NA (Figure 3.1a). In single provenance tests, the difference between 540 and 720 PPM CO_2 in NA was significant in P3, P25 and P26 (Figure 3.1a, Appendix II)

No significant CO_2 effect on WUE was found in combined ANOVA (Table 3.2), but WUE of seedlings at 540 and 720 PPM CO_2 generally were 32% and 35% higher than that of seedlings at ambient CO_2 (Figure 3.1b). In spite of this lack of significance when provenances were grouped, significant difference was observed between CO_2 levels when the provenances were considered individually. The southwest provenances generally had higher WUE values than that of north-shore ones at 540 and 720 PPM CO_2 (Figure 3.1b).

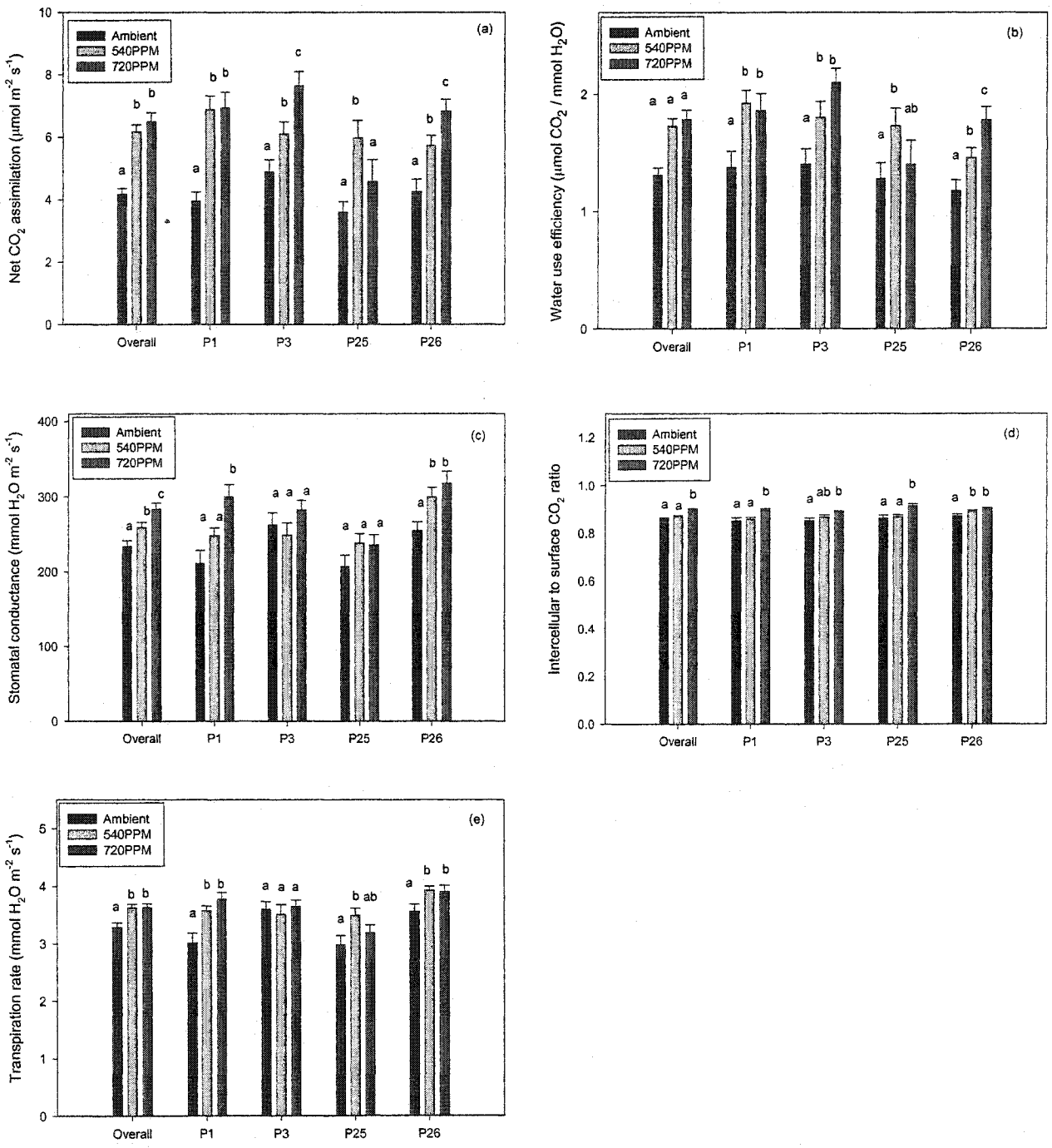

Figure 3.1 NA (a), WUE (b), g_s (c), Ci/Ca (d) and E (e) (means ± 1 SE) of four provenances of trembling aspen in October 2001. Measurements were taken 30 days after the seedlings were exposed to three levels of CO_2 concentrations: ambient, 540 and 720 PPM.

Note: Within one group of bars (e.g., P1), different letters on top of the bar represent significant differences within this group ($p<0.05$).

Table 3.2 ANOVA results for the effects of CO_2 enrichment on NA, WUE, g_s, Ci/Ca and E after 30 days (October 2001) of exposures to: ambient, 540 PPM and 720PPM CO_2.

		NA		WUE		g_s		Ci/Ca		E	
Source	Df	MS	F	MS	F	MS	F	MS	F	MS	F
C	2	113.53	6.27 **	4.86	3.13	45350	12.90 ***	0.031	8.20 **	2.76	5.29 **
B(C)	6	18.11	3.24 **	1.56	2.74 **	3514	0.92	0.004	1.90	0.52	1.35
δ	0	N/A		N/A		N/A		N/A		N/A	
P	3	22.64	1.57	1.36	2.27	37832	3.80 *	0.005	4.42	3.17	3.17 *
CP	6	9.58	1.38	0.52	0.62	6661	1.24	0.001	0.37	0.69	1.79
BP	18	5.58	1.39	0.57	1.81	3821	1.00	0.002	1.33	0.38	1.08
F(P)	8	10.22	1.90	0.66	1.15	8696	1.61	0.003	0.94	0.67	1.89
CF	16	5.39	1.34	0.58	1.84 **	5397	1.41	0.003	1.84 **	0.35	0.99
BF	48	4.022	2.79 ***	0.31	2.09 ***	3840	1.25	0.002	2.00 ***	0.36	1.54 **
Error	108	1.44		0.15		3080		0.001		0.23	

Note: 1. NA: net CO_2 assimilation; WUE: photosynthetic water use efficiency; g_s: leaf stomatal conductance; Ci/Ca: ratio of intercellular to leaf surface CO_2 concentration; E: leaf transpiration rate.
2. Asterisks indicate significance level: * = $p \leq 0.10$, ** = $p \leq 0.05$, *** = $p < 0.01$.

The 540 and 720 PPM treatments significantly increased g_s (11% and 21% respectively) compared to the ambient CO_2 (Table 3.2 and Figure 3.1c) in combined ANOVA. In single provenance ANOVA, the difference was not significant in P3 and P25. The difference between 540 PPM and ambient CO_2 was only significant in P26 (Figure 3.1c).

The Ci/Ca was generally higher in seedlings grown under elevated CO_2, but only the effect of 720 PPM $CO_2$was statistically significant for all the provenances in combined ANOVA (Table 3.2 and Figure 3.1d). The effect of 540 PPM CO_2 was only significant in P26 in single provenance ANOVA (Figure 3.1d).

The both CO_2 enrichments significantly increased E (about 10%) in comparison to the ambient CO_2 treatment in combined ANOVA (Table 3.2 and Figure 3.1e). There were no significant differences between the 540 and 720 PPM CO_2 (Figure 3.1e). In single provenance ANOVA, there was no significant change of E in P3 at both CO_2 enrichment treatments ($p > 0.05$), and the increase of E in P25 at 720 PPM was also not significant.

Gas exchange in Phase 2 (April 2002)

After 60 days of exposures to CO_2 treatments, all gas exchange variables except transpiration rate (E) were significantly increased by CO_2 elevations when tested by combined ANOVA (Table 3.3). It showed no significant provenance effects or family effects or significant CO_2-family interactions on any of the gas exchange variables ($p > 0.05$, Table 3.3). However, the block and family interaction were generally highly significant ($p<0.01$) for Net CO_2 assimilation (NA), photosynthetic water use efficiency (WUE), stomatal conductance (g_s) and transpiration rate (E).

In combined ANOVA, NA of seedlings grown at 540 and 720 PPM CO_2 were 28% and 22% higher, respectively, than that of seedlings grown under ambient CO_2 ($p < 0.05$, Table 3.3 and Figure 3.2a). NA of the 540 PPM treatment was significantly higher than the 720 PPM treatment (Figure 3.2a). In single provenance ANOVA, this difference was not significant in P1, P3 and P25 (Figure 3.2a).

WUE of seedlings at 540 and 720 PPM CO_2 were also 41% and 23% higher, respectively, than that of seedlings under ambient CO_2 in combined ANOVA ($p < 0.05$, Table 3.3 and Figure 3.2b). The 540 PPM CO_2 resulted in significantly higher WUE than the 720 PPM CO_2 (Figure 3.2b). In single provenance ANOVA, the difference in WUE between 720 PPM and ambient CO_2 was not significant ($p > 0.05$) in P1 and P26 (Figure 3.2b). However, the pattern of response was different among provenances: WUE in the 540 PPM treatment was significantly higher than the 720 PPM treatment in the two southwestern provenances but not in the north shore provenances (Figure 3.2b).

Over all the provenances, 540 PPM CO_2 treatment resulted in a significant (15%) decrease in g_s, but not 720 PPM CO_2 (Table 3.3 and Figure 3.2c). The differences were

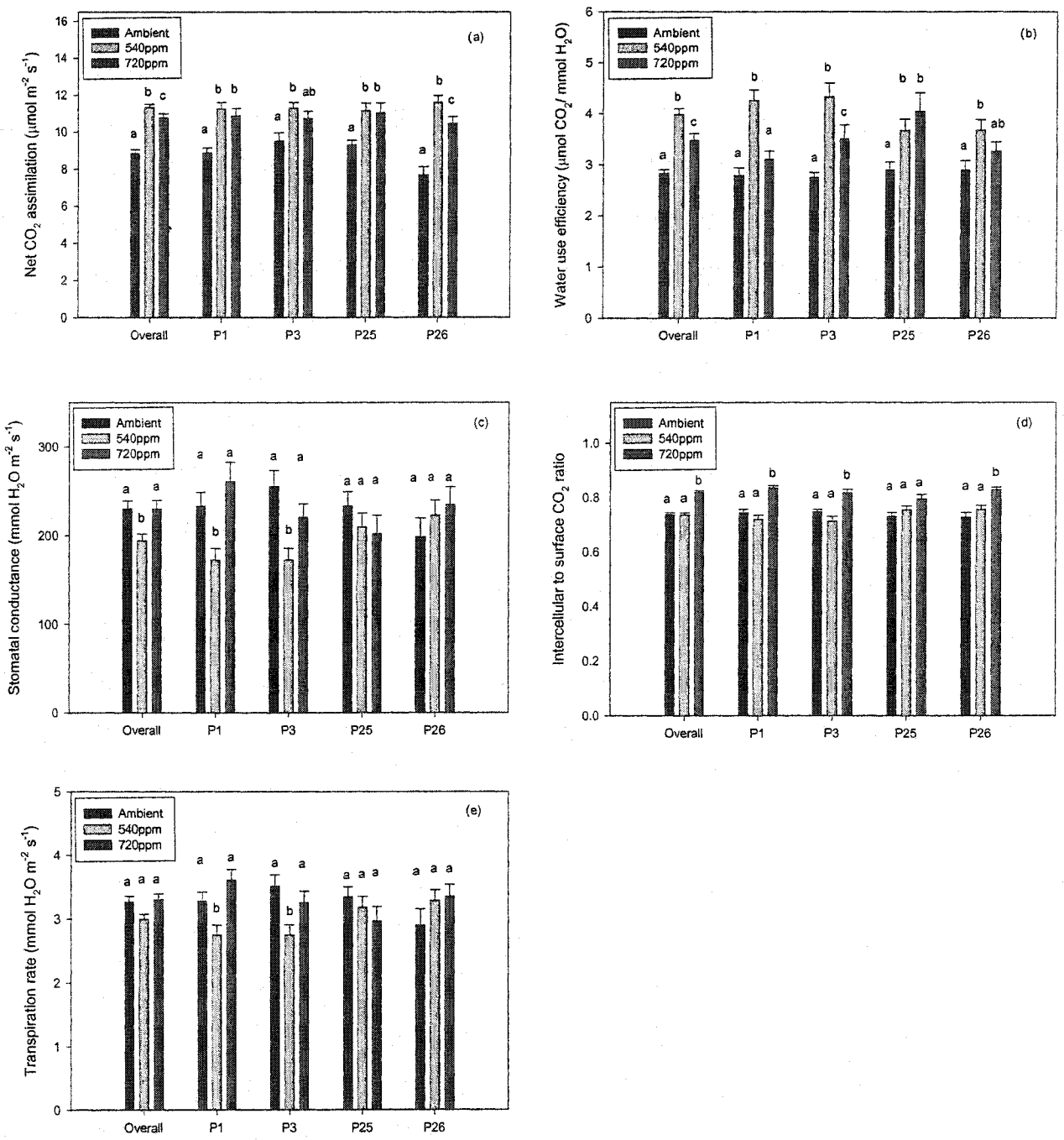

Figure 3.2 NA (a), WUE (b), g_s (c), Ci/Ca (d) and E (e) (means ± 1 SE) of four provenances of trembling aspen in April 2002. Measurements were taken 60 days after the seedlings were exposed to three levels of CO_2 concentrations: 380, 540 and 720 PPM.

Note: Within one group of bars (e.g., P1), different letters on top of the bar represent significant differences within this group ($p<0.05$).

significant only in the two southwestern provenances (Figure 3.2c). There were no significant differences in g_s between the 720 PPM and ambient CO_2 treatment in any provenance (Figure 3.2c).

Despite the g_s reductions in the 540 PPM CO_2 treatment, the overall intercellular to leaf surface CO2 ratio (Ci/Ca) was not significantly affected by the 540 PPM CO_2, but was significantly increased by 720 PPM (11%). A pattern was seen for all of the provenances when considered individually except P25 (Table 3.3 and Figure 3.2d).

The E of seedlings was not changed significantly by either 540 or 720 PPM CO_2 ($p > 0.05$, Table 3.3 and Figure 3.2e). In single provenance ANOVA, Southwestern provenances showed a significant reduction of E only by the 540 PPM CO_2 treatment (16% in P1 and 21% in P3), but not the 720 PPM CO_2 treatment. The north shore provenances did not show any significant responses to either CO_2 enrichment (Figure 3.2e).

Table 3.3 ANOVA results for the effects of CO_2 enrichment on NA, WUE, gs, Ci/Ca and E after 60 days (April 2002) of exposures to ambient, 540 PPM and 720 PPM CO_2.

		NA		WUE		g_s		Ci/Ca		E	
Source	Df	MS	F	MS	F	MS	F	MS	F	MS	F
C	2	122.35	24.13 ***	24.06	44.12 ***	30308	6.62 **	0.163	80.14 ***	2.00	2.12
B(C)	6	5.07	0.74	0.55	0.45	4577	0.52	0.002	0.42	0.94	1.11
δ	0	N/A		N/A		N/A		N/A		N/A	
P	3	4.21	1.96	0.85	0.38	544	0.03	0.002	0.19	0.02	0.01
CP	6	4.84	0.67	2.31	1.76	16259	2.26	0.007	1.92	1.93	2.67 *
BP	18	6.85	1.79 **	1.22	1.20	8830	1.18	0.005	1.14	0.84	1.07
F(P)	8	1.50	0.36	1.00	0.90	5357	0.91	0.004	1.16	0.61	0.91
CF	16	4.19	1.09	1.11	1.10	5859	0.78	0.003	0.78	0.67	0.84
BF	48	3.83	3.30 ***	1.01	1.46 **	7498	1.81 ***	0.004	1.27	0.80	1.91 ***
Error	108	1.16		0.69		4145		0.003		0.42	

Note: 1. NA: net CO_2 assimilation; WUE: photosynthetic water use efficiency; g_s: leaf stomatal conductance; Ci/Ca: ratio of intercellular to leaf surface CO_2 concentration; E: leaf transpiration rate.

2. Asterisks indicate significance level: * = $p \leq 0.10$, ** = $p \leq 0.05$, *** = $p < 0.01$.

Photosynthetic down–regulation

When all the seedlings were measured at a common CO_2 level (360 PPM), there was a marginal CO_2 effect on NA ($p=0.09$) when provenances were grouped in ANOVA (Appendix II). NA of the 720 PPM CO_2 was significantly lower (by 10%) than the 540 PPM and ambient CO_2 treatment in *post-hoc* test, while was no significant difference between the latter two ($p < 0.05$, Figure 3.3). No significant differences or other effects were found when provenances were considered individually in this down-regulation of NA at 720 PPM CO_2 (data not presented).

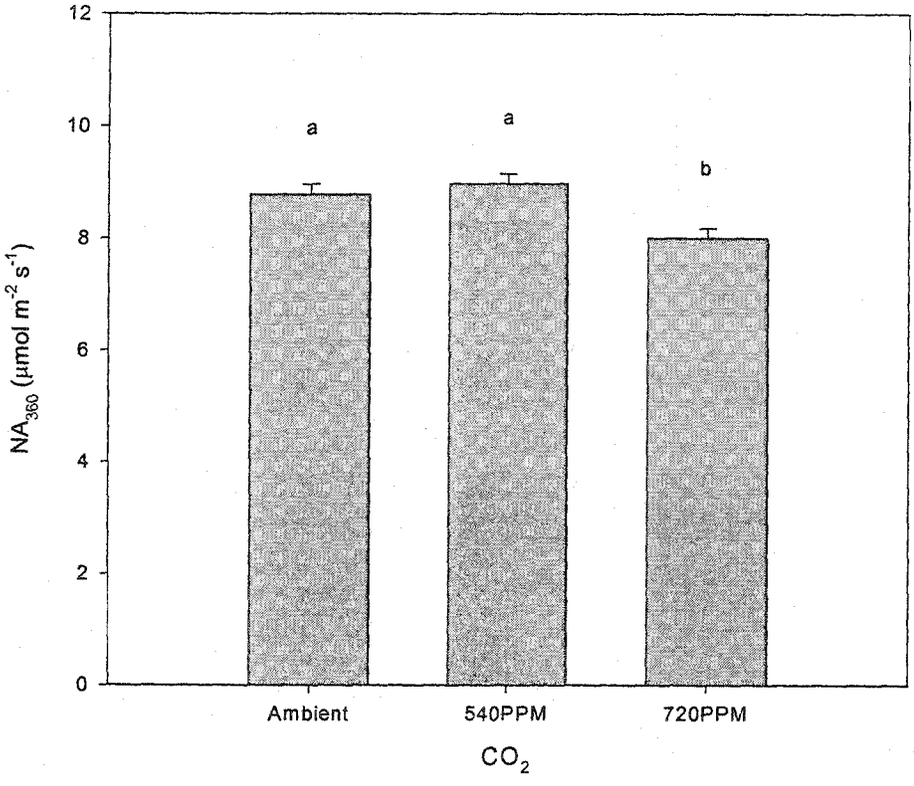

Figure 3.3 Net CO_2 assimilation (NA) of trembling aspen seedlings grown at three CO_2 levels for 60 days but measured at a common CO_2 concentration (360 PPM). Different letters on top of the bars indicate significant difference at 0.05.

Biomass

Provenance effects were significant for all of the biomass components in combined ANOVA, and significant block effects were found for all of the biomass components ($p<0.05$) except that the effect on leaf mass was marginal ($P<0.10$, Table 3.4). There were significant family effects for total, leaf and root biomass, but there were no significant CO_2 - family or block - family interactions for any of the biomass components (Table 3.4).

The 540 and 720 PPM CO_2 levels significantly increased the total biomass of seedlings by 30% and 12%, respectively, ($p < 0.05$, Table 3.4 and Figure 3.4a). The seedlings at 540 PPM CO_2 generally had higher total biomass than those at 720 PPM ($p < 0.05$), but this difference became insignificant statistically when the data were analyzed for individual provenance in univariate tests (Figure 3.4a). There were differences in response to CO_2 enrichments in total biomass between the two southwestern provenances and two north shore ones. In single provenance ANOVA, both 540 and 720 PPM CO_2 significantly increased the total biomass of two southwestern provenances (Figure 3.4a). In contract, the CO_2 enrichments did not affect the total biomass of two north shore provenances significantly (Figure 3.4a).

The overall shoot mass was increased 25% by 540 PPM CO_2 ($p < 0.10$), but there was no significant increase by 720 PPM CO_2 in combined ANOVA. However, there were differences in the response between provenances in single provenance ANOVA (Table 3.4 and Figure 3.4b). Within the two southwest provenances, shoot mass of P1 was enhanced 23% by 540 PPM, but not by 720 PPM CO_2, while the shoot mass of P3 were 51% and 39% higher, respectively, at 540 and 720 PPM than at ambient CO_2 treatment (Figure 3.4b). The north-shore provenances showed a similar weaker pattern but the differences were not significant ($p > 0.05$, Figure 3.4b). The results for stem mass were nearly identical with the pattern observed for shoot mass (Figure 3.4c).

The overall leaf mass generally did not response significantly to CO_2 elevations in combined ANOVA (Table 3.4). However in single provenance ANOVA, individual provenance showed up to 39% increase at 540 PPM CO_2 and 35% increase at 720 PPM CO_2 (P3, Figure 3.4d).

There were 46% and 23% increases in root mass by the 540 and 720 PPM CO_2 treatment, respectively, in comparison to ambient CO_2 ($p < 0.01$, Table 3.4 and Figure 3.4e). Root mass at 540 PPM CO_2 was significantly higher than that at 720 PPM CO_2 (Figure 3.4e). There were response differences between provenances in single provenance ANOVA tests. While both southwestern provenances showed significant increases in root mass in response to both CO_2 elevations, the two north shore provenances showed weaker insignificant responses to the 720 PPM CO_2 and the increase at 540 PPM CO_2 was only significant for P26 (by 55%) ($p < 0.05$, Figure 3.4e).

Table 3.4 ANOVA results for the effects of CO_2 enrichment on total, shoot, stem, leaf and root biomass of four trembling aspen provenances after 60 days of exposures in April 2002.

		Total		Shoot		Stem		Leaf		Root	
Source	Df	MS	F	MS	F	MS	F	MS	F	MS	F
C	2	2957	5.59 **	1090	3.57 *	333	3.59 *	233	3.06	466	8.93 ***
B(C)	6	529	3.29 ***	305	3.06 **	93	3.27 **	76	2.41 *	52	3.43 **
δ	0	N/A		N/A		N/A		N/A		N/A	
P	3	4769	9.50 ***	2488	9.52 ***	739	12.82 ***	526	5.98 **	381	6.91 ***
CP	6	307	3.89	194	3.39	59	2.56	43	2.16	29	2.79
BP	18	161	0.77	100	0.78	28	0.83	32	0.82	15	0.88
F(P)	8	323	2.53 **	152	1.79	28	0.95	72	2.67 **	39	3.08 **
CF	16	127	0.61	85	0.67	29	0.84	27	0.70	13	0.72
BF	48	209	1.14	128	1.08	34	1.06	39	1.14	17	1.28
Error	108	183		118		32		34		14	

Note: Asterisks indicate significance level: * = $p \leq 0.10$, ** = $p \leq 0.05$, *** = $p < 0.01$.

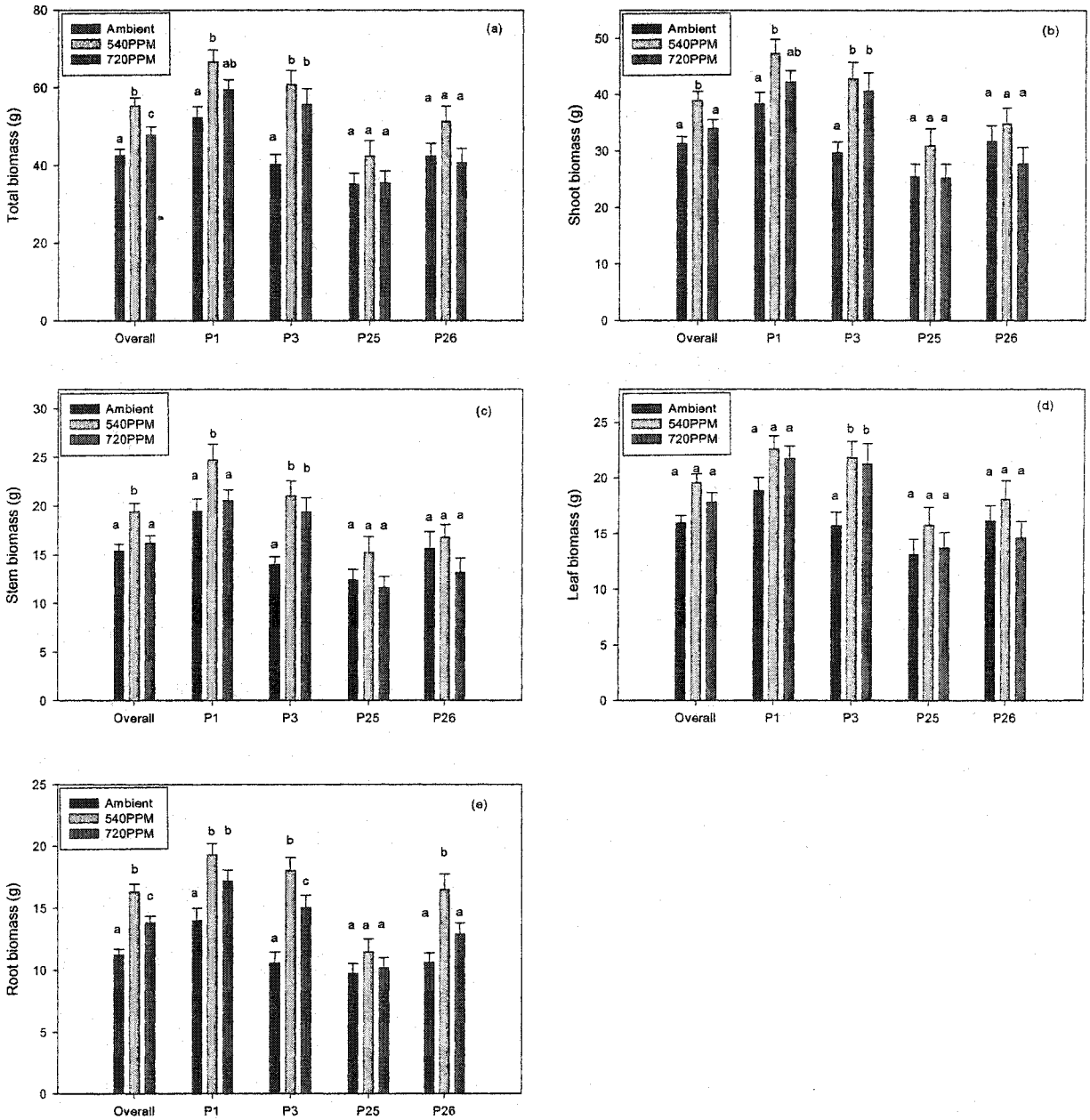

Figure 3.4 Total (a), shoot (b), stem (c), leaf (d) and root biomass (e) (means ± 1 SE) of four provenances of trembling aspen in April 2002. Measurements were taken after the seedlings were exposed 60 days to three levels of CO_2 concentrations: ambient, 540 and 720 PPM.

Note: Within one group of bars (e.g., P1), different letters on top of the bar represent significant differences within this group ($p<0.05$).

Biomass allocation

In contrast to the significant CO_2 effects on biomass components, CO_2 enrichments did not significantly affect stem mass to total mass ratio (SMR), leaf mass to total mass ratio (LMR), root mass to shoot mass ratio (RSR) or whole plant leaf area (LA) in combined ANOVA (Table 3.5). However, the root mass to total mass ratio (RMR) was increased about 10% by both CO_2 elevations ($p < 0.10$, Table 3.5 and Figure 3.5). There were also significant CO_2-family interactions for SMR and LMR, and marginal interactions for RMR and RSR (Table 3.5). There were provenance effects ($p < 0.10$) and family effects ($p < 0.05$) on LA, and block-family effects were also significant ($p < 0.05$, Table 3.5). There were marginal block effects on SMR, RMR and RSR ($p < 0.10$, Table 3.5).

In single provenance ANOVA tests, P26 showed significant increases (32% and 38%) in RMR at 540 and 720 PPM CO_2, respectively (Figure 3.5c, Appendix II). Additionally, P26 showed increased RSR in response to both CO_2 elevations (Figure 3.5d).

Table 3.5 ANOVA results for the effects of CO_2 enrichment on SMR, LMR, RMR, RSR and LA of four provenances trembling aspen after 60 days of exposures in April 2002.

		SMR		LMR		RMR		RSR		LA	
Source	Df	MS	F	MS	F	MS	F	MS	F	MS	F
C	2	0.0104	1.46	0.0087	1.75	0.0225	3.49 *	0.0830	2.56	0.0925	1.51
B(C)	6	0.0072	2.52 *	0.0050	1.37	0.0065	2.17 *	0.0324	2.43 *	0.0613	2.24 *
δ	0		N/A		N/A		N/A		N/A		N/A
P	3	0.0067	3.25	0.0041	1.07	0.0080	2.55	0.0428	3.08	0.2831	3.66 *
CP	6	0.0022	0.43	0.0019	0.30	0.0058	1.17	0.0243	1.18	0.0411	14.8
BP	18	0.0028	1.24	0.0036	1.52	0.0030	1.09	0.0133	1.07	0.0274	0.57
F(P)	8	0.0044	0.97	0.0071	1.38	0.0021	0.44	0.0093	0.47	0.0599	2.54 **
CF	16	0.0045	1.97 **	0.0051	2.15 **	0.0047	1.73 *	0.0198	1.59 *	0.0236	0.49
BF	48	0.0023	1.02	0.0024	1.10	0.0027	0.78	0.0124	0.75	0.0482	1.50 **
Error	108	0.0022		0.0022		0.0035		0.0165		0.0320	

Note: 1. SMR is stem mass to total mass ratio; LMR is leaf mass to total mass ratio; RMR is root mass to total mass ratio; RSR is root mass to shoot mass ratio; LA is whole plant leaf area (m^2).

2. Asterisks indicate significance level: * = $p \leq 0.10$, ** = $p \leq 0.05$, *** = $p < 0.01$.

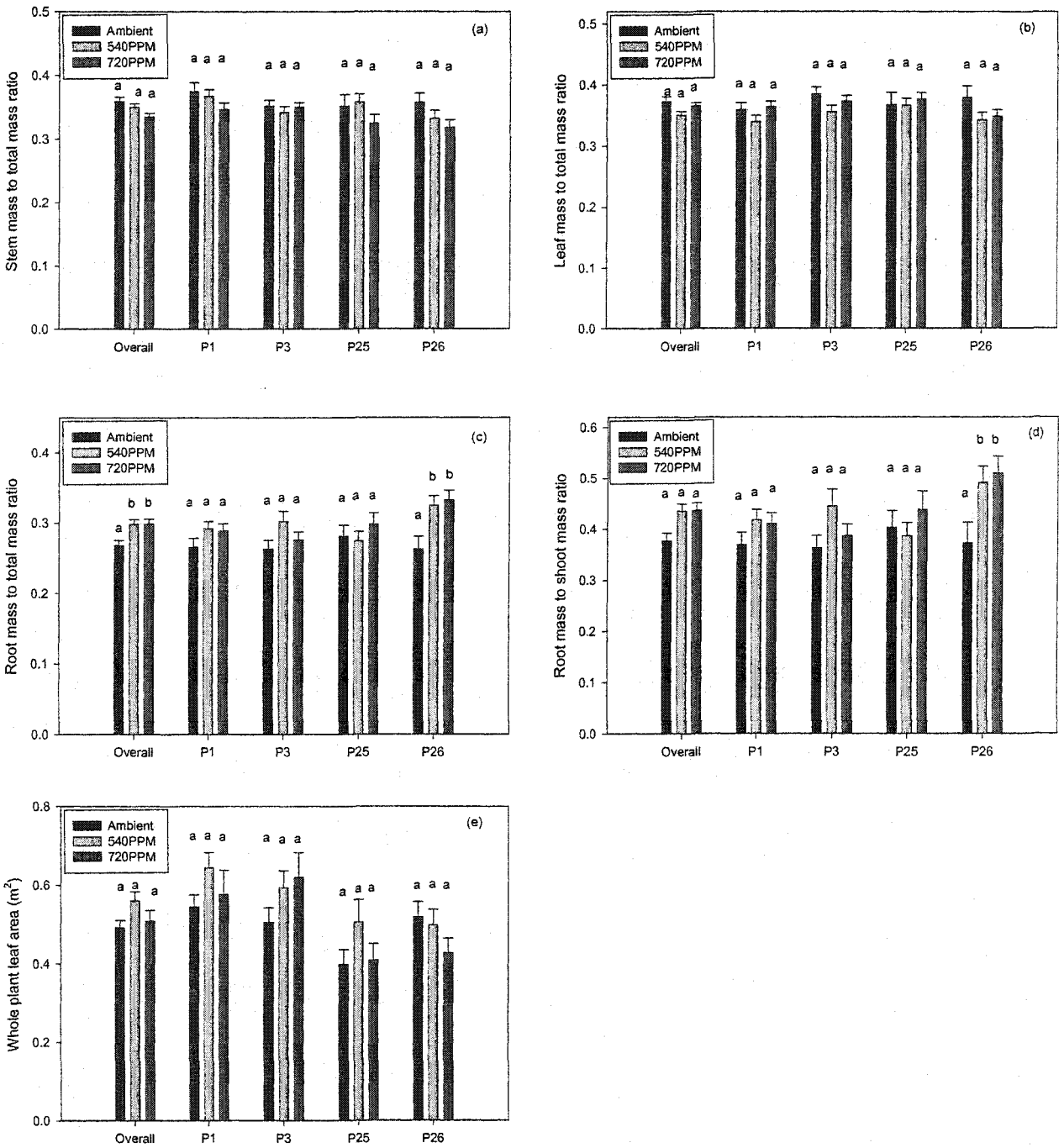

Figure 3.5 SMR (a), LMR (b), RMR (c), RSR (d) and LA (e) (means ± 1 SE) of four provenances of trembling aspen in April 2002. Measurements were taken after 60 days of exposures to three levels of CO_2 concentrations: ambient, 540 and 720 PPM.

Note: Within one group of bars (e.g., P1), different letters on top of the bar represent significant differences within this group ($p<0.05$).

Correlations between leaf gas exchange, biomass and climate variables

In April 2002, WUE was positively correlated with total biomass (r=0.19, p=0.10), and Ci/Ca was negatively correlated with total biomass (r=-0.20, p=0.10) at ambient CO_2 (Table 3.6). However, there were no other significant correlations between gas exchange variables and biomass at ambient or at 540 PPM CO_2. At 720 PPM CO_2, WUE was negatively correlated with total biomass (r=-0.60, p=0.03), while E and Ci/Ca were positively correlated with total biomass (r=0.22, p=0.07 and r=0.24, p=0.04 respectively).

At all three CO_2 levels, there were negative correlations between SLA and total biomass and positive correlations between LA and total biomass (Table 3.6). The correlations between SLA and total biomass were -0.41, -0.42 and -0.40, respectively, for the 380, 540 and 720 PPM CO_2 ($p<0.001$). The correlations between LA and total biomass were 0.71, 0.77 and 0.72, respectively, for the 380, 540 and 720 PPM CO_2 treatments ($p<0.0001$). WUE, E, and Ci/Ca were significantly correlated with nearly all biomass variables at 720 PPM CO_2 (Table 3.6c), but not at other two CO_2 concentrations (Table 3.6a and 3.6b). Furthermore, the polarity of the correlations between gas exchange and biomass variables was reversed from ambient and 540 PPM CO_2 to 720 PPM CO_2 (Table 3.6).

The simple regressions showed that gas exchange/growth variables were predicted by most climate variables under three CO_2 levels (Table 3.7). Moreover, monthly maximum temperatures were better predictors than minimum temperatures. For examples, monthly mean maximum temperatures in growing season (April to August) were closely related to gas exchange/growth variables. Monthly mean precipitation data were also good predictors of gas exchange/growth variables from April to July and from October to January. Furthermore, this relationship did not change over the three CO_2 concentrations (Table 3.7).

Table 3.6 Pearson correlations between gas exchange and growth traits for trembling aspen seedlings from northwestern Ontario under ambient (a), 540 PPM (b) and 720 PPM (c) CO_2 in April 2002.

a) Ambient

	A	WUE	E	Ci/Ca	g_s	SLA	Total	Shoot	Stem	Leaf	Root	LA
A	1.00											
WUE	-0.01	1.00										
E	0.60***	-0.78***	1.00									
Ci/Ca	0.07	-0.99***	0.80***	1.00								
g_s	0.55***	-0.77***	0.98***	0.81***	1.00							
SLA	-0.04	-0.17	0.10	0.15	0.09	1.00						
Total	-0.05	0.19	-0.18	-0.20*	-0.19	-0.41***	1.00					
Shoot	-0.04	0.19	-0.16	-0.18	-0.16	-0.43***	0.97***	1.00				
Stem	-0.11	0.16	-0.16	-0.17	-0.14	-0.29***	0.89***	0.91***	1.00			
Leaf	0.04	0.18	-0.14	-0.17	-0.14	-0.49***	0.88***	0.90***	0.64***	1.00		
Root	-0.07	0.15	-0.18	-0.17	-0.21*	-0.26**	0.80***	0.64***	0.59***	0.57***	1.00	
LA	0.00	0.07	-0.08	-0.07	-0.09	0.10	0.71***	0.74***	0.55***	0.80***	0.45***	1.00

b) 540 PPM

	A	WUE	E	Ci/Ca	g_s	SLA	Total	Shoot	Stem	Leaf	Root	LA
A	1.00											
WUE	0.11	1.00										
E	0.35**	-0.86***	1.00									
Ci/Ca	-0.09	-0.98***	0.84***	1.00								
g_s	0.28**	-0.85***	0.97***	0.86***	1.00							
SLA	-0.24**	-0.21*	0.10	0.21*	0.13	1.00						
Total	0.06	0.14	-0.12	-0.14	-0.16	-0.43***	1.00					
Shoot	0.07	0.14	-0.11	-0.15	-0.16	-0.41***	0.98***	1.00				
Stem	0.05	0.12	-0.10	-0.12	-0.13	-0.39***	0.93***	0.95***	1.00			
Leaf	0.09	0.15	-0.12	-0.16	-0.17	-0.38**	0.92***	0.94***	0.79***	1.00		
Root	0.02	0.10	-0.10	-0.10	-0.14	-0.40***	0.85***	0.73***	0.68***	0.69***	1.00	
LA	-0.01	0.10	-0.11	-0.13	-0.17	0.08	0.77***	0.79***	0.64***	0.87***	0.56***	1.00

c) 720 PPM

	A	WUE	E	Ci/Ca	g_s	SLA	Total	Shoot	Stem	Leaf	Root	LA
A	1.00											
WUE	0.21*	1.00										
E	0.33**	-0.76***	1.00									
Ci/Ca	-0.16	-0.99***	0.79***	1.00								
g_s	0.32**	-0.68***	0.96***	0.74***	1.00							
SLA	0.00	0.13	-0.11	-0.12	-0.12	1.00						
Total	0.03	-0.26**	0.22*	0.24**	0.17	-0.40***	1.00					
Shoot	0.05	-0.23**	0.22*	0.22*	0.17	-0.37***	0.99***	1.00				
Stem	0.07	-0.22*	0.22*	0.20*	0.18	-0.38**	0.95***	0.97***	1.00			
Leaf	0.02	-0.24**	0.20*	0.22**	0.15	-0.33**	0.97***	0.98***	0.90***	1.00		
Root	-0.04	-0.29***	0.19	0.26**	0.15	-0.42***	0.88***	0.79***	0.75***	0.78***	1.00	
LA	0.03	-0.14	0.12	0.13	0.07	0.26**	0.72***	0.75***	0.67***	0.80***	0.50***	1.00

Note: 1. Asterisks indicate significance level: * = $p \leq 0.10$, ** = $p \leq 0.05$, *** = $p < 0.01$.

2. There were 72 seedlings sampled for each CO_2 concentration.

3. The meanings of abbreviations see previous tables.

Table 3.7 The R-squares of simple regressions between monthly climate variables and gas exchange/growth measurement under ambient, 540 PPM and 720 PPM CO_2 for four provenances of trembling aspen from northwestern Ontario in April 2002. Only significant R-squares were presented.

	Ambient			540 PPM							720 PPM			
	WUE	Ci/Ca	SLA	A	WUE	E	Ci/Ca	g_s	SLA	Total	LA	A	SLA	Total	LA
JanMinT									0.87*						
FebMinT				0.80*											
MarMinT				0.80*											
AprMinT								0.80*			0.83*		0.88*		
MayMinT			0.84*								0.87*		0.93**		
JunMinT						0.81*		0.83*			0.93**		0.96**		
JulMinT			0.98***										0.81*		
AugMinT									0.85*						
SepMinT									0.91**						
OctMinT									0.85*						
NovMinT	0.90**	0.82*			0.89*		0.87*		0.80*					0.85*	0.93**
DecMinT									0.96**						
JanMaxT									0.99***						
FebMaxT			0.86*												
MarMaxT						0.87*		0.88*			0.92**		0.92**		
AprMaxT	0.82*	0.89*			0.91**	0.97**	0.91**	0.96**			0.98**		0.89*	0.90**	0.85*
MayMaxT	0.91**	0.93**			0.97**	0.97**	0.96**	0.95**		0.86*	0.95**		0.81*	0.96**	0.93**
JunMaxT	0.89**	0.92**			0.96**	0.97**	0.95**	0.95**		0.87*	0.96**		0.83*	0.97**	0.92**
JulMaxT	0.87*	0.91**			0.94**	0.97**	0.94**	0.96**		0.84*	0.97**		0.86*	0.95**	0.89*
AugMaxT	0.80*	0.86*			0.89*	0.95**	0.88*	0.95**			0.98**		0.91**	0.89*	0.81*
SepMaxT			0.83*								0.84*		0.89*		
OctMaxT			0.93**												
NovMaxT									0.91**						
DecMaxT									0.95**						
JanMnP	0.85*	0.91*			0.93**	0.98***	0.92**	0.97**			0.97**		0.87*	0.91**	0.87*
FebMnP												0.83*			
MarMnP															
AprMnP	0.90**	0.84*			0.92**		0.90**			0.88*				0.92**	0.95**
MayMnP	0.94**	0.89**			0.95**	0.86*	0.94**	0.82*		0.87*				0.94**	0.98**
JunMnP	0.90**	0.85*			0.93**	0.83*	0.91**			0.92**				0.96**	0.95**
JulMnP	0.95**	0.91**			0.97**	0.88*	0.96**	0.84*		0.87*				0.94**	0.98**
AugMnP									0.85*						
SepMnP				0.87*								0.89*			
OctMnP	0.84*	0.91**			0.91**	0.98**	0.91**	0.98***			0.95**		0.85*	0.85*	0.84*
NovMnP	0.86*	0.92**			0.93**	0.99***	0.93**	0.98***			0.95**		0.84*	0.88*	0.87*
DecMnP	0.85*	0.91**			0.92**	0.99***	0.92**	0.98***			0.96**		0.85*	0.88*	0.86*

Note: 1. Asterisks indicate significance level: * = $p \leq 0.10$, ** = $p \leq 0.05$, *** = $p < 0.01$.

2. The meanings of gas exchange and growth abbreviations see previous tables.

3. JanMinT means Janurary mean minimum temperature; JanMaxT means January mean maximum temperature; JanMnP means January mean precipitation; so were true for other climate variable abbreviations.

DISCUSSION

In Phase 1 of the experiment (autumn 2001), CO_2 elevations significantly increased net CO2 assimilation (NA) and photosynthetic water use efficiency of trembling aspen seedlings. These results are consistent with studies on the responses of leaf gas exchange to short term CO_2 elevation in higher plants (Lamber et al 1998). The overall 48% and 55% increases in NA by 540 and 720 PPM CO_2 are consistent to previous reports for woody plants (Curtis 1996; Curtis and Wang 1998). The result that seedlings grown at 540 and 720 PPM also had higher stomatal conductance (g_s) and transpiration rate (E) than those at ambient CO_2 is in contrast to the literature reporting that elevated CO_2 treatment reduces g_s (Curtis 1996). The reason for the increase seen in this study is not clear. Furthermore, the responses varied with provenances. For example, P3 and P25 failed to show a significant response to elevated CO_2, while P1 and P26 increased significantly. This genetic variation in response to CO_2 may have implications to the genetic structure of trembling aspen under future climate with a higher atmospheric CO_2 concentration.

In Phase 2 (spring 2002), the seedlings were again exposed to the same CO_2 treatments for 60 days after a dormant period of 120 days. This second CO_2 enrichment significantly changed the leaf gas exchange properties of the seedlings. The 28% and 22% overall increases in NA at 540 and 720 PPM CO_2 were moderate compared to the long term CO_2 exposure on poplar clones in field conditions (Curtis and Wang 1998; Kubiske et al 1997, 1998). The results of Phase 2 were in agreement with findings that the response of NA in greenhouse grown plants was intermediate between growth-chamber and open-top-chamber grown plants (Curtis 1996). Although almost a threefold difference in the magnitude of CO_2 enhancement of NA were reported between several poplar genotypes (Kalina and Ceulemans 1997; Wang et al 2000), no

significant differences in the response of NA to CO_2 elevation were found between families or between provenances in either phase of this study. In contrast to Phase 1 (autumn 2001), provenances showed differences in g_s in Phase 2. For example, the g_s of southwest provenances (P1 and P3) grown at 540 PPM CO_2 were significantly lower than at ambient CO_2. The g_s of north-shore provenances (P25 and P26) grown at 540 PPM CO_2 did not change compared to that of seedlings grown at ambient CO_2. This result is similar to the varying responses of trembling aspen genotypes in the literature (Wang et al 2000). On the other hand, the lack of a reduction in g_s for all provenances at 720 PPM CO_2 agrees with the conclusions of Curtis (1996) and Curtis and Wang (1998).

The lack of significant provenance and family effects in gas exchange in combined ANOVA at both CO_2 elevations could be due to several reasons. In Phase 1, CO_2 treatments were delayed until the seedlings started to set buds because of delays of installation of CO_2 generating equipment. There may have been insufficient time for the provenances to show differences. In Phase 2, any differences between provenances or families may not have been detected due to the low light conditions (270 to 320 PAR) in the greenhouses and the small pot sizes relative to tree size might have limited the development of provenance or family differences. The masking effect of unfavorable environment to genetic differences has been reported in other studies (Curtis and Wang 1998). Additionally, the complex experiment design resulted in small error terms (2 seedlings per family and 3 families per provenance), which reduced the sensitivity of F-tests.

There are contradictory results in the literature regarding photosynthetic down-regulation in response to CO_2 enrichment in woody plants under optimal conditions (Curtis and Wang 1998; Aber et al 2001; Herrick and Thomas 2001). However, plants tend to acclimate to long term CO_2 elevations (>50 days) (Curtis 1996; Aber et al 2001). Photosynthetic down regulation had been

reported for some plant species (Centritto and Jarvis 1999; Klus et al 2001; Kubiske et al 2002). In this study, trembling aspen seedlings showed a 10% photosynthetic down-regulation in photosynthetic capacity in response to the 720 PPM CO_2 treatment, but not to the 540 PPM treatment. This difference in photosynthetic down-regulation may be an explanation for the smaller increase of NA at 720 PPM CO_2 than at 540 PPM CO_2. This difference tends to suggest that photosynthetic down-regulation may be a threshold response that only occurs when CO_2 concentration reaches a certain level. In addition, no provenance differences were found in this photosynthetic acclimation. Because the 60-day CO_2 exposure was short compared to most field studies (Curtis 1996; Kubiske et al 2002), it may not have been long enough to reveal any difference between provenances.

The 540 PPM and 720 PPM CO_2 enrichments increased the total biomass of the seedlings by 30% and 12%, respectively. The relative increase by 720 PPM CO_2 was similar to the 18% increase of shade intolerant species under sub-optimal conditions (Kerstiens 2001). The smaller total biomass enhancement at 720 PPM compared to 540 PPM CO_2 in this study may be related to photosynthetic down-regulation only having occurred at 720 PPM but not at 540 PPM CO_2. Consequently, the actual photosynthesis was higher at 540 PPM than at 720 PPM CO_2 (Figure 3.2a). Other environmental factors may also have contributed, such as interactions between CO_2 and temperature. The daytime high temperature inside the 720 PPM CO_2 greenhouse was generally 2°C higher, and the high temperature lasted longer (3 hour) than in the other two greenhouses (Figure 10, Appendix I). This higher temperature may have resulted in stress causing reduced daily photosynthetic production and increased respiration in the 720 PPM CO_2 treatment.

There were between-provenance differences in the response of biomass to CO_2 elevations. The southwestern provenances responded positively to CO_2 enrichments in most of the biomass components, while the north shore ones did not except for root mass of P26 at 540 PPM. This finding is consistent with previous reports on genetic variation in biomass enhancement. Previous studies have reported increases in biomass enhancement for some trembling aspen genotypes (Ceulemans et al 1995; Curtis 1996; Lindroth et al 2000; Wang et al 2000), but not in others (Kubiske et al 1998; Lindroth et al 2000). The genetic mechanism underlying these differential biomass responses remains unclear (Wang et al 2000). The contrasting responses of southwestern and north shore provenances raised the question: whether the two southwestern provenances would perform better in the future increased CO_2 environment than the two north shore ones. Although consistent with the short-term CO_2 results, longer field CO_2 treatment experiments would be required to test this hypothesis. This study suggests that genetic variation of trembling aspen in response to CO_2 elevations existed at provenance level, but not at family level. Different provenances displayed different responses to CO_2 elevations in both gas exchange and biomass (Figures 3.1-3.4), while no significant CO_2 and family interactions were found. This finding may have implications to the future genetic composition of trembling aspen. For instance, the two southwestern provenances might flourish in the future climate associated with CO_2 elevation.

The CO_2 enrichment generally did not affect biomass allocations in this study. Only a north shore provenance (P26) showed increases in RMR and RSR in response to CO_2 elevations. However, significant CO_2 and family interactions were found in SMR, LMR, RMR and RSR. Lindroth et al (2000) reported CO_2 and genotype interactions in RSR of trembling aspen, but not the LMR. The SMR and LMR generally decreased while RMR and RSR increased in response to

elevated CO_2. The increases of photosynthate allocation to roots under elevated CO_2 were strongly affected by other environmental factors (Curtis and Wang 1998). The pot size could restrict nutrient availability to plants. Such environmental constraints may have interacted with CO_2. Therefore, the biomass allocation results should be interpreted with caution.

Significant block * family interactions were found for most gas exchange variables but not for biomass and biomass allocation variables (Table 3.2, 3.3, 3.4 and 3.5). This result showed gas exchange variables were more sensitive to environmental conditions than biomass and biomass allocation, and different genotypes had different sensitivities to the environment because the setup of blocks was intended to balance hetero-environmental conditions that possibly existed inside the greenhouses.

There were no consistent correlations between leaf gas exchange and biomass at the three CO_2 levels. However, WUE, E and Ci/Ca were significantly correlated to biomass variables under 720 PPM CO_2 conditions, but not under ambient and 540 PPM CO_2. For example, WUE was positively correlated with total biomass at ambient CO_2, but the relationship took a sharp turn to negative at 720 PPM CO_2 (Table 3.6). In addition, the polarity of correlations between gas exchange and biomass variables was reversed from ambient and 540 PPM CO_2 to 720 PPM CO_2. It is possible that gas exchange variables could be predictors of biomass as atmospheric CO_2 concentration increases, but that this situation will be reversed when a threshold value is reached. This finding showed the adaptation process of trembling aspen seedlings to the new gas exchange environment. However, this experiment was only a short term exposure, and longer term CO_2 exposure is required to corroborate this finding. On the contrary, the leaf properties of seedlings, i.e., SLA and LA, maintained stable correlations with total biomass. This suggests leaf traits may be better predictors of biomass than gas exchange variables.

The regression analyses between gas exchange and monthly climate variables showed gas exchange traits of provenances were predicted by many climate variables under ambient CO_2 and elevated CO_2 concentrations. No similar studies on such relationship could be found in the literature. However, this finding suggests that the gas exchange of these provenances still could be predicted by local climates even if the atmospheric CO_2 increases in near future. The close relationship between gas exchange and local climatic conditions suggests that aspen provenances may have adapted to the local environment and that such adaptation will likely remain in the future. Moreover, any changes in the local environmental conditions are likely to shift the genetic structure of trembling aspen. The lack of significant correlations between biomass and the local climates of the provenances also suggests that biomass production is more plastic than gas exchange variables and are more sensitive to the current growing conditions than the local climates of the provenances. However, the different responses between biomass and gas exchange warrant future studies.

In conclusion, CO_2 enrichment affected leaf gas exchange and biomass characteristics of trembling aspen in northwestern Ontario, but different provenances responded differently. The two southwestern provenances were more responsive than the two north shore provenances. These differences could result in an intraspecific distribution shift within this region. Future aspen improvement should exploit this genetic variation by selecting seed sources that are able to take advantage of future atmospheric CO_2 concentration within northwestern Ontario. However, the different responses to CO_2 elevation might have been confounded with the effect of other environmental factors due to the limited capacity of the greenhouses in controlling the environmental conditions. For example, the temperature difference in the greenhouse of 720 PPM CO_2 was higher than the other two greenhouses, and high temperatures generally lasted

longer. While a high temperature is generally associated with CO_2 elevation, such interaction is likely to modify trees responses to elevated CO_2 (Aber et al 2001; Kerstiens 2001; Pooter and Perez 2001). Therefore, the results of this study should be used or interpreted with caution.

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APPENDIX I: TYPICAL DIURNAL PATTERN OF ENVIRONMENTAL CONDITIONS IN THE GREENHOUSES

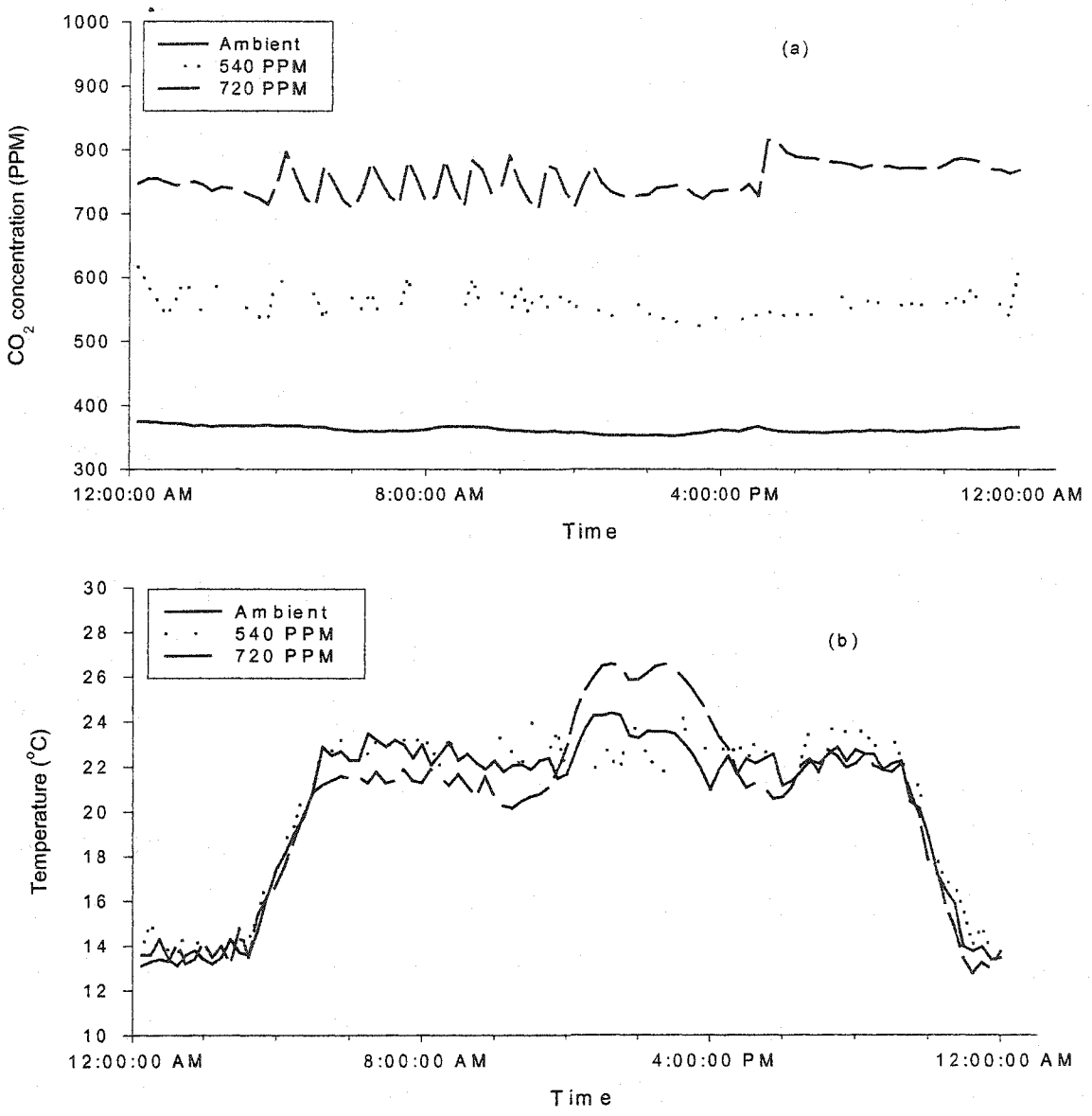

Figure A.1 Typical diurnal variations of CO_2 (a) and air temperatures (b) for three CO_2 treatment settings in greenhouses.

APPENDIX II: SUPPLEMENTAL TABLES

Table A.2 Net CO_2 assimilation (NA), water use efficiency (WUE), stomatal conductance (g_s), intercellular to leaf surface CO_2 ratio (Ci/Ca), transpiration rate (E), height (H) and root collar diameter (RCD) of trembling aspen seedlings from four provenances in northwestern Ontario in three measurements.

Variable	Date of Measurement	Provenance							
		P1		P3		P25		P26	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE
NA	Aug-01	5.27	0.53	2.07	0.23	4.18	0.55	3.67	0.36
	Oct-01	3.96	0.29	4.89	0.39	3.60	0.33	4.26	0.40
	Apr-02	8.88	0.27	9.52	0.44	9.31	0.25	7.68	0.45
WUE	Aug-01	2.74	0.18	2.44	0.16	1.96	0.27	3.08	0.32
	Oct-01	1.38	0.14	1.40	0.13	1.28	0.14	1.18	0.09
	Apr-02	2.79	0.15	2.75	0.09	2.89	0.16	2.89	0.19
g_s	Aug-01	189.8	36.3	44.8	4.5	189.9	23.2	95.1	18.2
	Oct-01	211.1	17.4	262.2	16.1	206.7	15.4	254.4	11.6
	Apr-02	232.9	15.5	254.9	18.4	233.2	15.9	198.1	21.2
Ci/Ca	Aug-01	0.788	0.015	0.752	0.014	0.833	0.019	0.833	0.019
	Oct-01	0.852	0.012	0.851	0.011	0.862	0.012	0.870	0.012
	Apr-02	0.742	0.014	0.746	0.009	0.729	0.015	0.729	0.015
E	Aug-01	2.08	0.24	0.82	0.07	2.30	0.17	1.38	0.19
	Oct-01	3.02	0.17	3.60	0.14	2.98	0.17	3.56	0.12
	Apr-02	3.28	0.14	3.51	0.18	3.35	0.16	2.90	0.25
H	Aug-01	74.83	1.65	80.32	1.67	50.83	4.65	71.99	1.62
	Oct-01	84.12	1.77	83.19	1.69	69.17	2.65	77.84	2.38
RCD	Aug-01	5.32	0.12	5.36	0.12	4.05	0.32	5.12	0.11
	Oct-01	6.58	0.07	6.23	0.16	5.64	0.14	6.14	0.16

Note: There were 216 seedlings total in August 2001, and 72 seedlings in October 2001 and April 2002.

Table A.3 Total, stem, leaf and root biomass of trembling aspen seedlings from four provenances in northwestern Ontario in April 2002 harvest.

Biomass Components	Provenance							
	P1		P3		P25		P26	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Total	52.33	2.84	40.24	2.59	35.23	2.70	42.40	3.30
Stem	19.48	1.26	13.98	0.82	12.39	1.10	15.63	1.73
Leaf	18.87	1.20	15.70	1.23	13.12	1.36	16.14	1.39
Root	13.98	1.02	10.57	0.89	9.72	0.81	10.63	0.75

Note: There were 72 seedlings totally measured.

Table A.4 Net CO_2 assimilation (NA), water use efficiency (WUE), stomatal conductance (g_s), ratio of intercellular to leaf surface CO_2 concentration (Ci/Ca), transpiration rate (E) (means ± SE) of four provenances of trembling aspen after 30 days of CO_2 exposures in October 2001.

Provenance		Ambient CO_2	540 PPM CO_2	720 PPM CO_2
Overall	NA	4.18 (0.18) a	6.17 (0.22) b	6.50 (0.29) b
	WUE	1.31 (0.06)	1.73 (0.06)	1.79 (0.08)
	g_s	233.6 (8.0) a	258.8 (7.0) b	283.8 (8.1) c
	Ci/Ca	0.859 (0.005) a	0.870 (0.004) a	0.899 (0.003) b
	E	3.29 (0.08) a	3.63 (0.06) b	3.63 (0.07) b
P1	NA	3.95 (0.29) a	6.88 (0.44) b	6.93 (0.50) b
	WUE	1.38 (0.14) a	1.92 (0.11) b	1.86 (0.14) b
	g_s	211.1 (17.4) a	248.4 (10.0) a	299.7 (16.5) b
	Ci/Ca	0.852 (0.012) a	0.858 (0.007) a	0.895 (0.006) b
	E	3.02 (0.17) a	3.57 (0.09) b	3.78 (0.11) b
P3	NA	4.89 (0.39) a	6.09 (0.39) b	7.64 (0.46) c
	WUE	1.40 (0.13) a	1.80 (0.14) b	2.10 (0.12) b
	g_s	262.2 (16.1) a	248.9 (16.3) a	282.2 (13.0) a
	Ci/Ca	0.851 (0.011) a	0.865 (0.009) ab	0.887 (0.005) b
	E	3.60 (0.19) a	3.51 (0.17) a	3.65 (0.11) a
P25	NA	3.60 (0.33) a	5.97 (0.56) b	4.57 (0.71) a
	WUE	1.28 (0.14) a	1.73 (0.15) b	1.40 (0.20) ab
	g_s	206.7 (15.4) a	238.2 (12.3) a	235.4 (13.8) a
	Ci/Ca	0.862 (0.012) a	0.867 (0.009) a	0.915 (0.008) b
	E	2.98 (0.17) a	3.49 (0.13) b	3.19 (0.14) ab
P26	NA	4.25 (0.40) a	5.72 (0.32) b	6.83 (0.37) c
	WUE	1.18 (0.09) a	1.46 (0.08) b	1.78 (0.11) c
	g_s	254.4 (11.6) a	299.5 (12.8) b	317.8 (15.8) b
	Ci/Ca	0.870 (0.008) a	0.889 (0.006) b	0.901 (0.005) b
	E	3.56 (0.12) a	3.93 (0.07) b	3.91 (0.11) b

Note: 1. Within a row, values followed by different letters are significantly different at $p = 0.05$.

2. NA ($\mu mol\ CO_2\ m^{-2}\ s^{-1}$); WUE ($\mu mol\ CO_2$ / $mmol\ H_2O$); g_s ($mmol\ H_2O\ m^{-2}\ s^{-1}$); E ($mmol\ H_2O\ m^{-2}\ s^{-1}$)

Table A.5 Net CO_2 assimilation (NA), water use efficiency (WUE), stomatal conductance (g_s), intercellular to leaf surface CO_2 ratio (Ci/Ca), transpiration rate (E) (means ± SE) of four provenances of trembling aspen after 60 days of CO_2 exposures in April 2002.

Provenance		Ambient CO_2	540 PPM CO_2	720 PPM CO_2
Overall	NA	8.84 (0.20) a	11.33 (0.18) b	10.77 (0.21) c
	WUE	2.83 (0.07) a	3.98 (0.12) b	3.47 (0.13) c
	g_s	229.8 (9.1) a	194.0 (7.7) b	229.3 (9.9) a
	Ci/Ca	0.746 (0.007) a	0.735 (0.008) a	0.818 (0.006) b
	E	3.26 (0.10)	2.99 (0.08)	3.30 (0.09)
P1	NA	8.88 (0.27) a	11.25 (0.35) b	10.87 (0.39) b
	WUE	2.79 (0.15) a	4.25 (0.21) b	3.10 (0.16) a
	g_s	232.9 (15.5) a	172.1 (13.1) b	260.7 (22.1) a
	Ci/Ca	0.742 (0.014) a	0.719 (0.015) a	0.834 (0.009) b
	E	3.28 (0.14) a	2.75 (0.16) b	3.61 (0.17) a
P3	NA	9.52 (0.44) a	11.30 (0.31) b	10.73 (0.39) ab
	WUE	2.75 (0.09) a	4.34 (0.26) b	3.50 (0.27) c
	g_s	254.9 (18.4) a	172.2 (13.1) b	220.3 (15.2) a
	Ci/Ca	0.746 (0.009) a	0.713 (0.018) a	0.817 (0.013) b
	E	3.51 (0.18) a	2.75 (0.16) b	3.26 (0.18) a
P25	NA	9.30 (0.25) a	11.15 (0.40) b	11.03 (0.54) b
	WUE	2.89 (0.16) a	3.67 (0.22) b	4.04 (0.37) b
	g_s	233.2 (15.9) a	209.3 (15.4) a	201.9 (20.5) a
	Ci/Ca	0.729 (0.014) a	0.754 (0.015) ab	0.794 (0.017) b
	E	3.35 (0.16) a	3.18 (0.17) a	2.97 (0.22) a
P26	NA	7.68 (0.45) a	11.6 (0.37) b	10.46 (0.36) c
	WUE	2.89 (0.18) a	3.67 (0.21) b	3.26 (0.18) ab
	g_s	198.0 (21.2) a	222.5 (16.8) a	234.4 (20.2) a
	Ci/Ca	0.726 (0.018) a	0.755 (0.015) a	0.828 (0.010) b
	E	2.90 (0.25) a	3.29 (0.17) a	3.36 (0.18) a

Note: 1. NA (μmol CO_2 m^{-2} s^{-1}); WUE (μmol CO_2 / mmol H_2O); g_s (mmol H_2O m^{-2} s^{-1}); E (mmol H_2O m^{-2} s^{-1}).
2. Within a row, values followed by different letters are significantly different at $p = 0.05$.

Table A.6 ANOVA results of the effect of CO_2 elevation on net CO_2 assimilation (NA) of four provenance trembling aspen from northwestern Ontario grown at ambient, 540 PPM and 720 PPM CO_2 but measured at a common CO_2 concentration (360 PPM).

Source	Df	MS	F	
C	2	18.7459	3.64 *	
B(C)	6	5.1519	1.1	
δ	0			
P	3	5.6591	2.12	
CP	6	2.1716	0.57	
BP	18	4.6835	1.26	
F(P)	8	3.3585	1.17	
CF	16	2.8635	0.77	
BF	48	3.7173	4.65 ***	
Error	213	0.7988		

Note: Asterisks indicate significance level: * = $p \leq 0.10$, ** = $p \leq 0.05$, *** = $p < 0.01$.

Table A.7 Total, shoot, stem, leaf and root biomass (g) (means ± SE) of four provenances of trembling aspen after 60 days of CO_2 exposures in April 2002.

Provenance		Ambient CO_2	540 PPM CO_2	720 PPM CO_2
Overall	Total	42.6 (1.59) a	55.3 (2.09) b	47.8 (2.05) c
	Shoot	31.3 (1.25) a	39.0 (1.58) b	34.0 (1.59) a
	Stem	15.4 (0.70) a	19.4 (0.87) b	16.2 (0.78) a
	Leaf	15.4 (0.68)	19.5 (0.80)	17.8 (0.85)
	Root	11.2 (0.47) a	16.3 (0.64) b	13.8 (0.54) c
P1	Total	52.3 (2.84) a	66.6 (3.11) b	59.5 (2.61) ab
	Shoot	38.4 (2.12) a	47.3 (2.51) b	42.3 (2.02) ab
	Stem	19.5 (1.26) a	24.7 (1.64) b	20.6 (1.10) a
	Leaf	18.9 (1.20) a	22.6 (1.17) a	21.7 (1.14) a
	Root	14.0 (1.01) a	19.3 (0.90) b	17.2 (0.90) b
P3	Total	40.2 (2.59) a	60.9 (3.60) b	55.7 (4.03) b
	Shoot	29.7 (1.95) a	42.8 (2.92) b	40.7 (3.22) b
	Stem	14.0 (0.82) a	21.0 (1.53) b	19.4 (1.42) b
	Leaf	15.7 (1.23) a	21.8 (1.48) b	21.2 (1.84) b
	Root	10.6 (0.89) a	18.0 (1.08) b	15.0 (1.01) c
P25	Total	35.2 (2.70) a	42.4 (3.94) a	35.5 (3.07) a
	Shoot	25.5 (2.19) a	31.0 (3.06) a	25.3 (2.43) a
	Stem	12.4 (1.10) a	15.2 (1.61) a	11.6 (1.14) a
	Leaf	13.1 (1.36) a	15.7 (1.63) a	13.7 (1.40) a
	Root	9.7 (0.81) a	11.5 (1.05) a	10.2 (0.81) a
P26	Total	42.4 (3.30) a	51.3 (3.86) a	40.7 (3.73) a
	Shoot	31.8 (2.78) a	34.8 (2.81) a	27.8 (2.95) a
	Stem	15.6 (1.73) a	16.8 (1.30) a	13.2 (1.49) a
	Leaf	16.1 (1.39) a	18.1 (1.67) a	14.6 (1.52) a
	Root	10.6 (0.75) a	16.5 (1.28) b	12.9 (0.91) a

Note: Within a row, values followed by different letters are significantly different at $p = 0.05$.

Table A.8 SMR, LMR, RMR, RSR and LA (means ± SE) of four provenances of trembling aspen after 60 days of CO_2 exposures in April 2002.

Provenance		Ambient CO_2	540 PPM CO_2	720 PPM CO_2
Overall	SMR	0.36 (0.007)	0.35 (0.006)	0.34 (0.006)
	LMR	0.37 (0.008)	0.35 (0.005)	0.37 (0.005)
	RMR	0.27 (0.007) a	0.30 (0.007) b	0.30 (0.007) b
	RSR	0.38 (0.015)	0.44 (0.014)	0.44(0.015)
	LA	0.49 (0.019)	0.56 (0.024)	0.51 (0.028)
P1	SMR	0.37 (0.014) a	0.37 (0.011) a	0.35 (0.010) a
	LMR	0.36 (0.011) a	0.34 (0.010) a	0.36 (0.009) a
	RMR	0.26 (0.013) a	0.29 (0.010) a	0.29 (0.010) a
	RSR	0.37 (0.024) a	0.42 (0.021) a	0.41 (0.021) a
	LA	0.54 (0.031) a	0.64 (0.039) a	0.58 (0.062) a
P3	SMR	0.35 (0.009) a	0.34 (0.009) a	0.35 (0.007) a
	LMR	0.39 (0.012) a	0.36 (0.010) a	0.37 (0.009) a
	RMR	0.26 (0.012) a	0.30 (0.015) a	0.28 (0.011) a
	RSR	0.36 (0.024) a	0.44 (0.033) a	0.39 (0.022) a
	LA	0.50 (0.037) a	0.59 (0.044) a	0.62 (0.063) a
P25	SMR	0.35 (0.018) a	0.36 (0.012) a	0.32 (0.014) a
	LMR	0.37 (0.020) a	0.37 (0.011) a	0.38 (0.011) a
	RMR	0.28 (0.016) a	0.27 (0.013) a	0.30 (0.016) a
	RSR	0.40 (0.033) a	0.39 (0.026) a	0.44 (0.036) a
	LA	0.40 (0.038) a	0.51 (0.058) a	0.41 (0.041) a
P26	SMR	0.36 (0.014) a	0.33 (0.012) a	0.32 (0.012) a
	LMR	0.38 (0.019) a	0.34 (0.013) a	0.35 (0.011) a
	RMR	0.26 (0.018) a	0.32 (0.014) b	0.33 (0.014) b
	RSR	0.37 (0.041) a	0.49 (0.032) b	0.51 (0.033) b
	LA	0.52 (0.039) a	0.50 (0.040) a	0.43 (0.037) a

Note: 1. SMR is stem mass to total mass ratio; LMR is leaf mass to total mass ratio; RMR is root mass to total mass ratio; RSR is root mass to shoot mass ratio; LA is whole plant leaf area (m^2).

2. Asterisks indicate significance level: * = $p \leq 0.10$, ** = $p \leq 0.05$, *** = $p < 0.01$.

Table A.9 Single provenance ANOVA results for Chapter 3.

		October 2001									
		A		WUE		g_s		Ci/Ca		E	
Source	Df	MS	F	MS	F	MS	F	MS	F	MS	F
P1											
C	2	52.23	16.33 ***	1.61	5.17 ***	35667	8.81 ***	0.010	6.70 ***	2.80	9.13 ***
Error	51	3.20		0.31		4049		0.001		0.31	
P3											
C	2	34.35	11.07 ***	2.18	7.15 ***	5069	1.22	0.006	3.87 **	0.09	0.25
Error	51	3.10		0.31		4167		0.001		0.36	
P25											
C	2	25.60	4.64 **	0.98	1.97	5475	1.58	0.015	8.47 ***	1.17	3.12 **
Error	51	5.52		0.50		3454		0.002		0.38	
P26											
C	2	30.09	12.50 ***	1.64	9.86 ***	19126	5.82 ***	0.004	5.73 ***	0.75	3.78 ***
Error	51	2.41		0.17		3286		0.001		0.20	
		April 2002									
		A		WUE		g_s		Ci/Ca		E	
Source	Df	MS	F	MS	F	MS	F	MS	F	MS	F
P1											
C	2	29.24	13.85 ***	10.73	19.37 ***	37023	6.86 ***	0.067	22.34 ***	3.38	7.74 ***
Error	51	2.11		0.55		5395		0.003		0.44	
P3											
C	2	14.95	5.67 ***	11.33	12.32 ***	31067	6.97 ***	0.051	14.89 ***	2.71	5.03 ***
Error	51	2.64		0.92		4456		0.003		0.54	
P25											
C	2	19.21	6.21 ***	6.18	4.88 **	4805	0.88	0.019	4.44 **	0.64	1.05
Error	51	3.09		1.27		5468		0.004		0.61	
P26											
C	2	73.45	26.31 ***	2.76	4.11 **	6193	0.90	0.049	12.49 ***	1.08	1.41
Error	51	2.79		0.67		6851		0.004		0.77	
		Total		Shoot		Stem		Leaf		Root	
Source	Df	MS	F	MS	F	MS	F	MS	F	MS	F
P1											
C	2	918.1	6.24 ***	363.5	4.07 **	137.2	4.16 **	68.7	2.80 *	129.0	8.08 ***
Error	51	147.0		89.2		33.0		24.5		16.0	
P3											
C	2	2071.2	9.62 ***	896.3	6.60 ***	246.2	8.15 ***	204.9	4.83 **	253.3	14.17 ***
Error	51	215.4		135.9		30.2		42.4		17.9	
P25											
C	2	300.4	1.55	185.9	1.55	65.7	2.15	34.1	0.88	14.6	1
Error	51	193.9		120.3		30.6		38.8		14.5	
P26											
C	2	587.8	2.47 *	226.3	1.55	60.9	1.47	54.5	1.29	156.0	8.58 ***
Error	51	238.2		146.0		41.4		42.2		18.2	
		SMR		LMR		RMR		RSR		LA	
Source	Df	MS	F	MS	F	MS	F	MS	F	MS	F
P1											
C	2	0.0037	1.55	0.0030	1.55	0.0037	1.69	0.0126	1.44	0.047	1.26
Error	51	0.0024		0.0019		0.0022		0.0087		0.038	
P3											
C	2	0.0005	0.40	0.0039	2.01	0.0071	2.38	0.0314	2.40 *	0.0652	1.49
Error	51	0.0013		0.0019		0.0030		0.0131		0.0438	
P25											
C	2	0.0057	1.39	0.0006	0.16	0.0026	0.64	0.0127	0.68	0.0625	1.62
Error	51	0.0041		0.0038		0.0041		0.0186		0.0386	
P26											
C	2	0.0071	2.31	0.0071	1.87	0.0264	6.20 ***	0.0994	4.41 **	0.0409	1.52
Error	51	0.0031		0.0038		0.0043		0.0225		0.0268	

Note: Asterisks indicate significance level: * = $p \leq 0.10$, ** = $p \leq 0.05$, *** = $p < 0.01$.